BIOCHEMICAL, ENVIRONMENTAL AND MEDICINAL EXPLORATION OF *EUPHORBIA PROSTRATA* AITON AND *EUPHORBIA GRANULATA* FORSSK

Ph.D. THESIS

BY

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DECLARATION

The materials contained within this thesis are my original work and have not been previously submitted to this or any other university.

MOHAMMAD PARVEZ

CERTIFICATE OF APPROVAL

This Dissertation entitled **"BIOCHEMICAL, ENVIRONMENTAL AND MEDICINAL EXPLORATION OF *EUPHORBIA PROSTRATA* AITON AND *EUPHORBIA GRANULATA* FORSSK"** submitted by Mohammad Parvez S/O Mian Abdul Qahar is hereby approved and recommended as partial fulfillment for the award of Degree of Doctor of Philosophy in Biotechnology.

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DEDICATION

This dissertation is dedicated to my beloved parents for a lot of prayers and to my brothers and sisters for their unending payers and support.

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All praises for Almighty **ALLAH**, The Omnipotent, Omnipresent, the Creator, the most Merciful, the Gracious, the Compassionate, the Beneficent and the Sole Source of Wisdom and Knowledge. It was Almighty **ALLAH** who gave me the courage and enabled me to achieve this goal.

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ABSTRACT

Euphorbia granulata Forssk and *Euphorbia prostrata* Aiton are two medicinal plants belonging to family euphorbiaceae. The present study was aimed at biochemical, environmental and medicinal exploration of these two plants.

Biochemically the two plants and their parts were investigated for proximate composition, important phytochemicals and mineral elements. Nutritionally both plants were found with almost identical and excellent quantities of crude fibers (18.3 ±0.68 to 20 ± 0.49), proteins (17.5 ±0.52 to 18.3 ± 0.18) and carbohydrates (39.64 ±0.29 to39.9 ±1.32) however *E. prostrata* was comparatively more nutritious than *E. granulata*. Similarly more lipids were noted for *E. granulata* compared to *E. prostrata*. Vertical distribution in plant parts showed comparatively more protein and lipids in seeds, more carbohydrates in stem and roots and more fibers in roots than other parts. Phytochemicals are medicinally important secondary metabolites. The composition of the two plants showed the presence of total phenolics, flavonoids, alkaloids, tannins, glycosides and saponins in good quantity. Comparing the two plants, showed high amount of phenolics, flavonoids and alkaloids in *E. granulata* however tannins and glycosides were found more in *E. prostrata*. Both plants contained equal amount of saponins. Leaves were found the highest contributor to the total phytochemicals quantified in whole plant. Macro and micro minerals in plants are important for the health of its consumers. The composition of *E. granulata* and *E. prostrata* revealed that the two plants were good source of most minerals. The plants contained micronutrients Fe, Mg, Mn, Zn, Mo and Se in sufficient amount to supply them well above the recommended dietary allowances (RDA). Only Cu was present in small amount. On the other hand only Cl was present in small amount while the rest of macronutrients (S, Ca, P, Na, N and K) were present in good dietary quantity.

Some plants have the unique ability of accumulating toxic heavy metals from contaminated soil called phyto-extractor. The phyto-extraction ability *E. granulata* and *E. prostrata* was checked for Hg, As, Pb, Cd, Co, Cr, Ag, Mo, Ni and Sn. Out of these tested heavy metals *E. granulata* was found to take up Mo, As and Ni actively while *E. prostrata* tend to extract Hg and Mo with bio-accumulation coefficient more than 1. The levels of heavy metals accumulated in the two plants were also evaluated for toxicity.

The observed concentrations in the two plants were not enough to produce toxicity specified by WHO/FAO and other authorities are mentioned in this dissertation.

The two plants exhibited pronounced effects on biochemical and physiological parameters. Diabetes mellitus (DM), coronary heart diseases (CHDs), hypertension, hepatitis, osteoporosis, and blood related diseases have posed challenge to the health care providers. Synthetic therapeutics is not only expensive but a number of adverse side effects are associated with them. Plants are possible sources of novel structural entities that could provide effective treatment to the prevailing diseases. Extracts of *E. granulata* and *E. prostrata* were noted to decrease the blood glucose level in normal rabbits more than in alloxan induced DM rabbits. The hypoglycemic effect was more pronounced with *E. granulata* than with *E. prostrata*. The two plants were also found effective in modulating the levels of serum lipid profile. The levels of TC, LDL and VLDL decreased with *E. granulata* extract, in normal, isoprenaline treated and diabetic rabbits but had no effect on the levels of HDL, TG or FFA. However in case of *E. prostrata*, additionally FFA levels also normalized with extract. The results of preventive groups revealed that both *E. granulata* and *E. prostrata* have the ability to minimize the myocardial cell injury caused by isoprenaline. ALT, AST, bilirubin, urea, albumin and total protein are biochemical markers that indirectly reflect the health of liver. Rabbits having hepatic injury when treated with extract of *E. granulata* had brought down significantly ($p < 0.01$) the levels of ALTand AST but had increased that of urea, TP and Alb. This was an indirect reflection of improved liver health. The impact of *E. prostrata* on these markers was not equally effective. In latter case, although ALT and AST decreased significantly ($p<0.05$) with extract dose but TP and Alb were not improved to any significant degree. The extract dose of both *E. granulata* and *E. prostrata,* before inducing liver injury was effective in minimizing the CCl_4 induced injury. The impact on bone health was monitored from the level of bone health markers (ALP, Ca and P levels). It was noted that the extract of *E. granulata* and *E. prostrata* had significant ($p > 0.05$) and similar to PTH effects on bone health profile. There were increased activities of ALP and high serum levels of Ca and P levels which were clear indications of positive impact on bone health. Three major electrolytes levels were monitored with different extract doses. The plant extract had no indication on influencing the levels of serum electrolytes. The two plants had also exhibited to have therapeutic effect in improving anemia in experimentally

induced animals. It was noted that with extract dose of *E. granulata* the values of Rbcs, Hb and PCV increased but no change was noted for MCH, MCHC and MCV. This represented that after treatment with extract Rbcs were produced having normal size and optimum Hb concentration. It was further investigated the plant extract had no effect in preventing the damage caused by PHZ. In case of *E. granulata* the ant anemic property was observed in both normal as well as in anemic rabbits but *E. prostrata* was effective in anemic subject only. Studying the impacts of the two plants on immunological parameters (Tlc, neutrophils, eosinophil, basophils, monocytes, lymphocytes and thrombocytes) unveiled that they have no role in modulating immune system.

The two plants were evaluated for antioxidant potential using DPPH free radical scavenging activity. *E. prostrata* whole plant exhibited more DPPH inhibition (67.37 ± 0.949%) than *E. granulata* (59.93 ± 1.058%) had. Stems of the two plants had equal activities while leaves of *E. prostrata* were more scavenging properties than *E. granulata*. However the case of roots was reversed of the leaves. Brine shrimp lethality bioassay results proved the non-cytotoxic nature of the two plants with high LC_{50} of 33.88 (E. granulate) and 25.7 mg / ml (*E. prostrata*). The plants were further checked for phytotoxicity using lemna bioassay and radish seeds germination and radical growth methods. The results suggested that *E. granulata* extracts had stimulant effect on *Lemna minor* growth but had no effects either on seed radish seed germination or radical growth. Furthermore phytotoxicity study revealed that *E. prostrata* had neutral activity towards *Lemna minor* and radish seeds germination. However the extracts had inhibitory effects on small doses but were stimulatory towards radical growth at large doses. Three methods were used to check the insecticidal potential of the two plants but both *E. granulata* and *E. prostrata* were ineffective against *Tribolium castaneum*. Antibacterial potential of the two plants were checked against eight pathogenic bacteria (*Klebsiella pneumonia, Bacillus cerus, Citrobacter frundii, Streptococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Escheritia coli* and *Serratia marcscens*). Of all the tested strains *Serratia marcscens* was found most sensitive while *Citrobacter frundii* (QUF-ATCC) the most resistant to both extracts. Similarly antifungal activities of the two plants were evaluated against four important fungal strains (*Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus,* and *Fusarium oxysporum*). Out of them *Aspergillus niger* was found sensitive to *E. granulata* while *Aspergillus flavus* to *E.*

prostrata. In combination with standard drug the extracts of both *E. granulata* and *E. prostrata* exhibited additive properties, against both bacterial strains as well as fungal strains. Antitumor potential were also tested using *Agrobacterium tumefaciens* on potato discs and carrot discs methods. The two had no tumor suppressing activities rather a 10% stimulatory effect on potato discs was noted for *E. granulata*.

PUBLICATIONS

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Phytochemistry of *Euphorbia granulata* Forssk in relation to its medicinal use. Pakistan Journal of Botany (Submitted)

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ABBREVIATIONS

EP *Euphorbia prostrata*

EG *Euphorbia granulata*

AC All carbohydrates

RS Reducing sugar

MS Monosaccharides

KS Ketose sugar

AS Aldose sugar

ND Not done

WP Whole plant

GAE Gallic acid equivalent

QE quercetin equivalent

PE Papaverine equivalent

TAE Tannic acid equivalent

DE Digitoxin equivalent

QSE Quillaja saponin equivalent

BDL Below detecting limit

BAC Bioaccumulation coefficient

PDI Possible daily intake

PHZ Phenylhydrazine

A 5 D After 5 days

NC Negative control

PC Positive control

PIN Percentage of insects on negative control side

PIP Percentage of insects on positive control side

P.Ex. Plant extract

CHAPTER 1

INTRODUCTION

1. Phytotherapy

Plants are the only autotroph and food producer for man, animals, microbes and without them life can't be sustained (Cutler & Cutler, 2000). Besides food and many other advantages, plants are the source of treating different ailments (Khan and Khan, 2007). As compared to synthetic medicine the use of plants are considered safe due to the presence many substances which are thought to be inactive but gives the plants high efficacy and safety compared to isolated compounds (Parekh & Chanda, 2007). The active principles for medicinal effect of plants are the presence of certain organic molecules called as secondary metabolites (Vaghasiya *et al*., 2011) which are in fact defense molecules of plants against herbivores but these molecules possess medicinal properties also(Kumar *et al*., 2009). About 4000 different compounds have been identified from plants and almost 200000 more are estimated to be discovered in future (Toghe *et al*., 2007). *Euphorbia granulata* (EP) and *Euphorbia prostrata* (EG) are medicinal plants used for different ailments without any distinction by the local population. The present study is aimed to study and compare the two plants in different situations. For this purpose phytochemistry, nutritional as well as medicinal properties of the two plants will be uncovered using bioassay techniques. Phytoextraction potential of the two species was also the focuss of the investigations.

2. Nutritional status

Food is the basic need of all living organisms. The basic purpose behind eating is getting calories, maintaining, growing, reproducing and multiplying of cells. A food must have proteins, carbohydrates, lipids, mineral elements, vitamins and water (Adnan *et al*., 2010). Man and animals cannot synthesis certain amino acids, fatty acids, and vitamins and must be supplemented readymade. The deficiency of these essential nutrients can leads to the diseased conditions and therefore the role of balance diet is important for good health (Hameed *et al*., 2008). This is why after the infectious diseases balanced

food supplement with important vitamins are recommended to overcome the loss of essential nutrients caused by infectious agent.

3. Mineral Elements

Proteins, carbohydrates and fats provide calories to the body (Hameed *et al*., 2008) while mineral nutrients or dietary minerals are important for normal physiological functions (Aslam *et al*., 2005). Some elements are considered as essential while the role of other elements is not defined e.g. Cd, Co and Ag (Saeed *et al*., 2010; Zafar *et al*., 2010). Seventeen elements have been observed as important for plants growth (Iwalewa *et al*., 2009) while for human and animals 21 mineral elements necessary (Omari, 2010). Out of 17 elements required by the plants 9 elements are macronutrients that includes, sulfur, carbon, potassium, calcium, nitrogen, phosphorus, hydrogen and magnesium while eight are micronutrients i.e. manganese, chlorine, Iron, zinc, nickel, molybdenum, copper and boron (Iwalewa *et al*., 2009). The macronutrients are sometime called as primary elements (Adeyeye, 2005).

Mineral nutrients are extremely important for the proper functioning of a number of proteins and hormones (Aslam *et al*., 2005). They are not consumed in the reaction but work as cofactors for enzymes (Iwalewa *et al*., 2009). The excess or absence of an element may produce certain diseases. Two criteria have been assigned to an element being essential or not. First: removing of the element may cause a disease and Second: re-adding the element to the diet relieves the disease symptoms (Zafar *et al*., 2010).

4. Phytochemistry

The word "Phyto" has Greek origin which means plant (Kumar *et al*., 2009) thus "phytochemistry" means plant chemistry. Phytochemicals are the substances found in edible fruits and vegetables that exhibit a potential for modulating human metabolism in a manner beneficial for the prevention of chronic and degenerative diseases (Sandhar *et al*., 2011).

Phytochemicals are defensive molecules against different diseases (Kumar *et al*., 2009). Their distribution in plants is not uniform but varies qualitatively and quantitatively from plant to plant and parts to part e.g. alkaloids are usually present in low concentration in

vegetative parts as compared to phenolic compounds and are present more concentrated in roots, seeds and fruits as compared to leaves. It is reported that 12000 have been identified and isolated so far (khan *et al*., 2011). Plants synthesize Phytochemicals to protect themselves from extinction against herbivores (Coolborn & Bolatito, 2010) by producing unpleasant smell (terpenoids), repulsive colors (tannins and quinines) and bad taste (Capsacin) while others are poisonous. Many of these biological molecules are high profile drugs and on this account plants are sometimes called as green factories (Vaghasiya *et al*., 2011). The branch of pharmaceutical science which is mainly involved with natural products (also called as botanicals, biochemicals or phytochemicals) from plants is called Pharmacognosy (Waksmundzka-Hajnos *et al*., 2008).

5. Phytoremediation and Heavy metals

Heavy metals are those elements which have atomic density of $4g/cm^3$ and are five to six time heavy than water. They are not required by the plants and are toxic to plants and animals even at low concentration (Duruibe *et al*., 2007). The presence of heavy metals in drinking water, soil or plant can result in serious health related problems because there is a narrow range between safe and toxic levels (Saeed *et al*., 2010). Heavy metal is considered as one of the important pollutants presents in our environments (Memon *et al*., 2001) because Plants have the ability to degrade organic compounds (Ya & Ramachandra, 2006; Aisien *et al*., 2010) but heavy metals and radionuclides cannot be degraded chemically or biologically (Prasad & Freitas, 2003). Once in the food chain the heavy metals keep on accumulating in the tissues and it is difficult to avoid their entrance into human body (Mudgal *et al*., 2010). Use of fossil fuels, mining, processing of metallic ores, municipal wastes, fertilizers, pesticides, and sewage are the main sources (Memon *et al*., 2001). Conventional way of cleaning is expensive and cumbersome while the use of plants for cleaning can be an economical and efficient method (Aisien *et al*., 2010).

6. Diabetes and Antidiabetic drugs

The “oldest disease” known to man (Tanko *et al*., 2008), Diabetes mellitus is a genetically transmitted complex and multifarious (Noor *et al*., 2008) endocrinal metabolic disorder and clinical syndrome (Rekha *et al*., 2010; Wadkar *et al*., 2008) with manifestation of higher level of blood glucose due to complete absence or decrease

secretion of insulin from β-cells of pancreas (Sunil, 2009; Priyanka *et al*., 2010). Aeretaeus (Greek physician) was the first to coin the word diabetes for the disease in 1st century. Wallis for the first time observed the sweet taste of urine from diabetic patient in 17th century. Dobson for the first time found sugar in urine of diabetic patient in 1755. 2-3% (200 million) peoples are suffering from the disease worldwide, 3000000 death occurs worldwide due to DM (Bnouham *et al*., 2006; Wadkar *et al*., 2008) and it is estimated that by the year 2025, 300 million people will be diabetic (Selvan *et al*., 2008). DM is estimated to kill more people than HIV and is at fourth major killer disease in developed countries (Bnouham *et al*., 2006).

7. Obesity and Antilipidemic drugs

Obesity is the result of excessive intake of energy rich food (especially those with high fats contents) and reduced utilization of this food energy due to reduced physical activities. It has also been observed that lipids abnormalities especially decreased levels of high density lipoprotein cholesterol (HDLC) and increased levels of low density lipoprotein cholesterol (LDLC) are the main contributing factors to coronary heart diseases CHD (Mansour *et al*., 2009). Myocardial infarction is reported to be the major health concern and cause of death globally (Khursheed *et al*., 2010). 70% of adult population suffers from atherosclerosis (Mansour *et al*., 2009). It is noted that atherosclerosis alone is responsible for more deaths than the deaths caused by all types of cancers (Amran *et al*., 2010). Every year 19million deaths occur worldwide due to atherosclerosis (Choi *et al*., 2010). According to some analysis 90% of atherosclerosis dies suddenly due to blocking of myocardial arteries and brain supply (Phadke, 2007).

8. Liver and Hepatoprotective drugs

Liver is one of the most important organs of the human and animal's body. Its importance can be judge from the fact that it is a multifunction organ performing the duty of storage, secretion, metabolism and detoxification (Ahsan *et al*., 2009).therefore liver is called as "the great chemical factory" of the body (Garba *et al*., 2009). A healthy liver is essential for overall health of a person but the fact is that liver is always exposed to a number of hazards including toxins, drugs, alcohols and viral infections (especially HCV and HBV) which often lead to liver damage and cirrhosis (Murugaian *et al*., 2009;

Sadeque and Begum, 2010). The liver injury caused by drugs, toxin and viruses is one of the challenges to scientists, health care providers and pharmaceutical industry. The drugs that caused liver failure and liver injury includes acetaminophen, chloroquine, isoniazid and rifampicin while the causative viruses are HCV and HBV (Saleem *et al*., 2008; Sadeque and Begum, 2010).

9. Plants and bone health

A normal level of calcium and phosphorus in blood reflect good health of bone and teeth (Al-Attar, 2010) and is mainly controlled by vitamin D and parathyroid hormones but due to low intake and under certain pathological condition and especially after menopause in female the blood calcium level decreases that leads to osteoporosis (Akhtar *et al*., 2007). Other pathologies that occur due to calcium phosphorus imbalance include paget´s disease, osteomalacia, osteitis fibrosa cystic, osteopetrosis and reckets. Plants can be a possible source of discovering a unique molecule has the potential of normalizing blood calcium and phosphorus level for a healthy bones and teeth.

10. Blood electrolytes

Electrolytes are substances which dissociates into positive and negative ions when dissolved in solvent and have the ability to conduct electricity. Sodium, potassium, chloride and bicarbonates are the four major electrolytes directly affecting the physiological characteristics of blood and other tissues. They are distributed in different ratios inside and outside the cells but a critical intracellular and extracellular physiological level are essential. Abnormal deviation from physiological values can leads to disease conditions like high blood pressure, hypotension, abnormal heartbeats and disturbance in the blood pH. Diuretics like frusemide, mannitol, ethacrinic and thiazides helps in relieving from fluid overload (Shivalinge *et al*., 2009).

11. Hematology and Immunology

Hematology deals with the study of structure, function, production, diseases, prognosis, diagnosis, and treatment of blood. The blood is the only liquid tissue. Blood is composed of two components, the liquid portion and the cellular portion (Cowan, 2012). The platelets help prevent bleeding by forming clot and disorder can results in failure to form a clot and ultimately lead death due to blood loss e.g. hemophilia. The red blood

cells supply oxygen to the tissue and remove the CO_2 from the tissues, abnormal RBCs can lead to fatal consequences e.g. sickle cell anemia (Lichtman *et al*., 2007). The WBCs are defense soldier that protect the body from invasion by pathogen and antigens. The factors that create negative impact on WBCs are called immuno-suppressants and those with positive impact are termed as immune enhancer or immuno-stimulant (Johnson *et al*., 2002).

12. Antibacterials

In pursuit of an effective, safe and economical antibacterial the first success in this direction was made by isolating the first antibiotic penicillin from *penicillium notatum* (Kotan *et al*., 2007). This discovery paved the way for the development of other antibiotics including cephalosporins, aminoglycosides, tetracyclines, chloramphenicol, macrolides, quinolones and many more. Non rationale use of antibiotics for the past few decades has evolved into a new concern of resistance (Nanasombat & Lohasupthawee, 2005). Bacteria are intelligently evolving and using different strategies to evade and combat the adverse effects of antibiotics. Some have developed resistant to one while other have developed resistance to many antibiotics and are called multidrug resistant strain (MDS). Methicillin resistant strain of *Staphylococcus aureus* (MRSA) are of especial concern (Aliero & Afolayan, 2006). To avoid the evolution of super bacteria researchers are looking for super molecules from different sources including plants, animals and microbes. (Kotan *et al*., 2007).

13. Antifungals

Unlike bacteria fungi have some unique features like cell wall structure, cytoplasmic organelles, lack of peptidoglycan and chlorophylls and ergosterol. These features both differentiate and make its resemblance with bacteria, plants and animals (Ryan & Ray, 2004). Treating fungal infection is a challenging task both for scientist and physician. Similarities between fungi and of host cell structure make the therapeutic agent to target functionality of the host cell also (Prescott *et al*., 2002). To avoid toxicity of these synthetic molecules the search for a safe solution can only be with plant or other living organisms (Ahmed & AbdelGaleil, 2005; Bi *et al*., 2008; Shin & Kang, 2002).

14. Oxidants and Antioxidant

Immense biochemical reactions takes place within living cells which accompanies a lot of energy shift from bond to bond. During these energy transfers high energy free radicals are produced one of them is the reactive oxygen species (ROS). ROS are high energy molecules and have the ability to damage the tissues. In the body there exists homeostasis, where free radicals are eliminated but under certain pathological conditions like diabetes mellitus (DM) the homeostasis is disturbed and a situation called oxidative stress arises where the ROS tends to damages the tissues (Patel *et al*., 2010; Ahmed *et al*., 2008). Different studies show that plants are rich in natural antioxidants (Elalla, 2009) and hence needs a thorough screening.

15. Tumor and Antitumors

Tumor is one of the challenges to the present medical research. Scientist are struggling hard to find an effective solution. For this purpose plants can be one of the arena to be screened for a powerful antitumor drug (Amara *et al*., 2008), using simple bench top bioassay technique like potato disc method (Ateeq-ur-Rehman *et al*., 2009).

16. Cytotoxicity

Cancer is uncontrolled cell divisions that proliferate at the expense or normal body cells and ultimately damage the surrounding tissues leading to malfunction of organ and ultimately death (Islam *et al*., 2009; Amara *et at*., 2008). After coronary diseases cancer is the leading cause of deaths and according to an estimate 10 million cases of different form of cancer are reported every year (Dahab & Afifi, 2007). Toxicity of chemotherapeutic agents to brine shrimps nauplii has direct correlation with human nasopharyngeal carcinoma (Fatima *et al*., 2009). Originally developed for the insecticidal potential of a product the brine shrimp lethality assay is now a day's an easy, time saving, effective and internationally accepted method for screening anticancer potential (Mutha & Shimp, 2010).

17. Phytotoxicity

Plants have evolved certain mechanism to save from hostile plants and animals e.g. insects. Plants produce certain secondary metabolites in its environments that not only save them from offensive organisms (plants, animals, microbes) but also eliminate

the hostile organisms from their proximity (Inderjit & Callaway. 2003; Yasmin *et al*., 2011). These metabolites are called phytochemicals and are usually present in roots, leaves, seeds, fruits, stem. They induce chlorosis, wilting and even death in the hostile plants (Lungu *et* al., 2011). Some have stimulatory effect on non-hostile and friendly plants (Yasmin *et al*., 2011). The phytochemicals that produces inhibitory or stimulatory impact on hostile or friendly plants are called allelochemicals and the phenomenon is called as allelopathy while the toxicity to other plants is called as phytotoxicity (Lungu *et* al., 2011; Khan *et al*., 2012; Gilani *et al*., 2010). Lemna bioassay, an easy to perform method, can be used to check plants for it toxic effects (Ateeq-ur-Rehman *et al*., 2009). Studies have shown that natural antitumor products inhibit lemna growth but other stimulates its growth. Therefore lemna phytotoxicity assay can be a rapid, easy, economical and simple bioassay and a tool for searching antitumor, phytotoxic and plant growth stimulant constituents (Hussain *et al*., 2010; Ayatollahi *et al*., 2010).

18. Insects and Insecticidals

Insects are the cause of many diseases in plants and animal and the cause of huge losses to the grains and seeds. Many insects like mosquitoes are vectors for communicating a number of diseases like malaria, dengue fever, filariasis, leishmaniasis, chickungunya , Japanese encephalitis, and yellow fever etc (Mallikarjun *et al*., 2010) while other are parasite itself on man and animal like tick, mites, flea and lice or *Pediculus humanus* (Yang *et al*., 2004). Many insect spoils the grains but *Tribolium castaneum, Trogoderma granarium, Spodoptera frugiperda, Leptinotarsa decemlineata, Bruchidius atrolineatus, Callosobruchus maculates, Oligonychus coffeae*, *Callosobruchus chinensis,* and *Callosobruchus rhodesianus* are the most destructive and widespread species (Alam *et al*., 2009; Nikkon *et al*., 2009; Hasan *et al*., 2006).

19. Resrearch Plants descriptions

Based on their medicinal properties, *Euphorbia granulata* a Forssk and *Euphorbia prostrata* an Aiton were selected for the present investigations. The plants were identified from department of Botany University of Peshwar, Pakistan. On line help from "Flora of Pakistan" was also used for plants identification. A voucher specimen was submitted to the said department for record and were numbered as, Bot-20012 (PUP) and

Bot-20013 (PUP) for *Euphorbia granulata* and *Euphorbia prostrata* respectively. Both plants belong to family Euphorbiaceae. *Euphorbia prostrata* is prostrate annual herb occurring all over in Indian subcontinent. It grows upto 30 cm in length. The plant is native to tropical and subtropical America and grows in sandy loam and calcareous soils. It most often occurs on footpaths, lawns and streams sides. *Euphorbia granulata* is a much branched prostrate annual or perennial herb with upto 20 cm long stem. It is found from North Africa to tropical Africa and to central Asia and North India. Occurs mostly in desert and semidesert habitate and can be found in areas where Euphorbia prostrata grows. Literature shows Pharmacognostic study of *Euphorbia prostrata* only (Sharma et al, 2012), however no biochemical, environmental and medicinal aspects of the two plants have been studied sofar.

Fig. 1. *Euphorbia granulata*.

Fig. 2. *Euphorbia prostrata*.

Fig. 3. *Euphorbia prostrata* (left), *Euphorbia granulata* (right).

CHAPTER 2

LITERATURE REVIEW

1. Proximate analysis

Plants are the only source of food for man, animals and microbes. There are 250000 plant species. However all of them are not important for provision of food. Some are nutritionally rich while others are not important as food. Analysis of medicinal plants for their nutritional value is important to get a clear picture of their nutritional status (Hussain *et al*, 2010). Following is the review of literature on the nutritional screening of medicinal plants.

Aberoumand (2010) after analyzing the nutritional contents of *Asparagus officinalis* and *Alocacia indica* found that there was good quantity of proteins (33%), carbohydrates (35%) and fats (3%) in *Asparagus officinalis* and high amount of carbohydrates (59%), lipids (3%) and fibers (23%) in *Alocacia indica*. Hence both the plants could be a good supplement for poor communities. Hussain *et al*. (2010) reported high carbohydrates, fats and fibers contents in *Zingiber officinalis* while *Hypericum perforatum*, *Allium sativum and Valeriana officinali* contained satisfactory levels of different elements and poor percentage of energy rich nutrients. *Zingiber officinale, Allium sativum* and *Parkia biglobosa,* used as food condiments, were investigated by Odebunmi *et al*. (2010) for proximate composition. It was seen that *Zingiber officinale* had highest moisture contents, followed by *Allium sativum* and *Parkia biglobosa.* It was further observed that with fermentation moisture and fat contents of *Parkia biglobosa* increased while protein contents decreased. Krishnamurthy & Sarala, (2010) stated that *Withania somnifera* collected from dry climate was rich in nutritional components and elemental composition. Kolawole *et al*, (2010) reported that the amount of protein (14%) and carbohydrates (51%) was high in tomatoes dried in light green colored light. Fats (1%) were high in tomatos dried in dark blue, fibers (25%) in light blue and ash (54%) in black colored dressing. Hence, different color frequencies affect biochemistry of plants.

Adnan *et al*, (2010) collected *Valeriana officinalis* and *Bupleurum falcatum* from humid region and *Forsskalea tenacissima*, *Lavandula angustifolia* and *Otostegia limbata* from sub-humid region. The analysis showed that macronutrients were in high amount in *Forsskalea tenacissima* while *Otostegia limbata* was rich in micronutrients this suggested that plants in sub-humid region were rich in nutrients as compared to those in humid region.

Hussain *et al*. (2010) evaluated that *Phlomis bracteosa* is nutritionally important due to its high contents of fats, fibers and energy value. They stated that *Dalbergia sissoo* and *Phlomis cashmeriana* had less nutritional values. Shehu & Aliero, (2010) compared the nutritional components of a healthy onion with a purple blotch-infected one. The diseased plant had low Protein, fats, carbohydrates, fibers and ash contents but they were rich in moisture contents (95%). The healthy plants showed all the nutritional contents in good amount but had reduced moisture contents (88%). The results suggested that infection reduced the nutritional value of plants. Seeds of *Lophira lanceolata* when analyzed by Lohlum *et al*. (2010) for their biochemical constituents and nutritional status showed that it had 49% fats, 30% protein, 12% carbohydrates, 8% fibers and 3% ash. The caloric value was 637kcal. Three essential amino acids leucine, isoleucine and lysine were also present. The proximate composition of macrolichen species *Ramalina hossei* when investigated showed high crude fibers (10.8%) and carbohydrates (60%) having energy value of 348cal/100gms (kumar *et al*., 2010). *Tamarindus indica*. Aberoumand (2010) reported that stem of *Asparagus officinalis* had 32.7% protein, 3.4% lipids, 34.7% carbohydrates, 10.7% ash and 18.5% fibers while fruits of *Momordica dioica* contained 5.4% protein, 3.2% lipids, 59.35% carbohydrates, 9.1% ash and 23% fibers.

Ezeibekwe *et al*. (2009) when analyzed *Plerotus tuberregium*, *Auricularia auricular* and *Plerotus squariosulus* for their nutritional status, reported that all the three species were rich in proteins (19.5-22.6%), carbohydrates (31.3-36.2%) and fats (1.3-3.7%). Besides fibers, ash and moisture were present to the extent of 4.5-6.5%, 5.8-7.2% and 11.2-12.8% each, respectively. *Moringa oleifera* contained 3% nitrogen and 19% protein as reported by Kawo *et al*. (2009). *Acalypha hispida*, *Acalypha marginata* and *Acalypha racemosa* were analyzed by Iniaghe *et al*. (2009) for proximate composition.

The results showed that proteins were 14%, 18%, 16%, carbohydrates 44.5%, 38%, 45%, fats 6%, 5.6%, 6.3%, moisture were 11%, 10.8%, 11.9% and ash were 10%, 15.6%, 13%, respectively, in each. Saidu & Jideobi, (2009) studied three plants from Minna (Saudi Arab) for proximate composition and found the results as: *Talinum triangulare* with 92% moistures, *Vernonia amygdalina* with 2.5% Ash and 0.7% of lipids while *Moringa oleifera* had 3% protein.

Habibullah *et al*. (2007) analyzed two varieties NM-92 and M1of mung for proteins, carbohydrates, fats, moisture, ash and fibers. They observed that NM-92 variety was rich in fats (2%), carbohydrates (59%) and fibers (7%) while M1 variety was rich in protein (24%), moisture (9%) and ash (4%). Energy value of NM-92 was 347kcal/100gms while that of M1 was 340kcal/100gms. *Tamarindus indica* contained 22% protein, 17% carbohydrates, 11% lipids and 4% fibers as reported by Yusuf *et al*. (2007). Abolaji *et al*, (2007) reported that *Parinari polyandra, Xylopia aethiopica* and *Blighia sapida* are often used during pregnancy in some parts of Nigeria. The analysis revealled that *Parinari polyandra* had highest protein contents (7%) and low in other nutrients while *Xylopia aethiopica* was rich in carbohydrates (55%), fats (9.5%), moisture (16%) and ash contents (4%) but poor in protein (only 2%). These plants were rich in mineral elements.

Odjegba & Fasidi, (2006) tested the effects of Cu, Ag, Zn, Hg, Ni, Cd, Pb and Cr on protein chlorophylls and starch contents of *Eichhornia crassipes*. The plants grown in soil with heavy metals showed reduction in starch, protein and chlorophyll contents while proline contents significantly increased. The increase or decreased in metal concentration had no effect on the overall results.Shad *et al*. (2002) found that ash and protein was highest in seeds of *Fagonia arabica* followed by leaves, shoots and roots. While fats and fibers were high in roots followed by shoots, leaves seeds. Seeds and leaves contained the highest moisture contents. Jimoh & Oladijii (2005) investigated that seeds of *Piliostigma thonningii* contained high protein contents, glycosides, anthraquinones, flavonoids, saponin and phenolics which imparted repulsive taste to seeds.

2. Phytochemicals

Phytochemicals are secondary metabolites of the plants that are produced as part of their defense strategy but these defensive molecules have potent medicinal value in human and animals (Qaisar *et al*., 2009). Medicinal plants have been analyzed qualitatively and quantitatively for phytochemicals to check their potential beneficial effects. Here are some of the recent studies carries out in different labs:

Methanolic and acetone extract of *Amaranthus asper* showed antibacterial and antioxidant potential (Jimoh *et al*., 2010). Subsequent screening for phytochemicals, revealed 0.3mg alkaloids, 7mg saponins and 9.25mg/100gm phytate of dry weight. Preliminary phytochemical analysis of *Digera muricata* indicated tannin, perpenes, saponins and flavonoids (Mathad & Mety, 2010). Odia *et al*. (2010) while screening *Cymbopogon citratus, Axonopus compressus* and *Eragrostis tremula* for phytochemicals reported contained cellulose, saponins and alkaloids in all the three plants. *Cymbopogon citratus* additionally contained inulin, tannin and lignin and *Axonopus compressus* also contained innulins and tannins.

Nuhu *et al*. (2009) reported the alkaloid composition of *Crotalaria microcarpa, Crotalaria naragutensis* and *Crotalaria retusa*, all contained pyrrolizidine alkaloids in their stem. Unsaturated pyrrolizidine alkaloid which is hepatotoxic was present only in *Crotalaria retusa*. Qaisar *et al*. (2009) noted that methanolic and dichloromethane extract from *Lantana camara* contained tannins, some cardioactive glycosides and triterpenes. In another study Edema *et al*. (2009) noted that polycyclic aromatic hydrocarbons were present at equal concentration in *Paspalum vaginatum* and *Ipomoea involucrate* that were not affected with oil. Leaves from *Caprica papaya, Pisidium guajava,* and *Vernonia amygdalina,* and stem bark from *Magnifera indica* were screened for the presence of phytochemicals (Ayoola *et al*., 2008). Alkaloids were absent in *Magnifera indica* and *Pisidium guajava,* anthraquinines were absent in *Pisidium guajava* and *Vernonia amygdalina* and cardiac glycosides absent in *Magnifera indica* while all the phytochemicals including flavonoids, terpenoids, saponins, tannins, alkaloids and anthrquinines were present in different ration in these plants.

Of the 60 medicinal plants, *Gentianopsis paludosa* was found with potential anti-tubercular activity. Subsequent screening for active molecules indicated the presence of seven compounds, including four compounds (1,7-dihydroxy-3,8-dimethoxyxanthone, 1,7,8-trihydroxy-3-methoxyxanthone, 1-hydroxy-3,7,8-trimethoxyxanthone and oleanolic acid) showing activity against growing mycobacterium (Fai *et al.,* 2008). Ramkumar *et al*. (2007) observed that ethanolic and hexane extracts of *Gymnema montanum* was active against *Salmonella typhi, Candida albican* and *Pseudomonas aeruginosa*. The extracts also showed antioxidant acitivity. Babu *et al*, (2007) when tested different organic and aqueous extracts from seven medicinal plants for antibacterial activity against phytopathogenic *Xanthomonas pathovars,* observed that antibacterial activity was more pronounced in organic solvent extract than in aqueous. Modnicki *et al*. (2007) detected apigenin, luteolin and aglycon in *Nepeta cataria*. They quantified flavonoids to be in the range of 0.3-0.46% of the dry weight. Phenolic acid was between 0.75 to 1.4% while rosmarinic acid was between 0.06 to 0.15%. It was noted that quantitatively phenolics and other flavanoids in *Nepeta cataria* and *Melissa officinalis* were in equal composition while rosmarinic acid was ten times less than in the other plants. Hajimehdipoor *et al.* (2007) identified secoiridoid dilactone, iridoid glycosides, nucleosides and secoirdoid glycosides in the n-butanol extracted fraction of *Swertia longifolia*.

Abutilon indicum, Acorus calamus, Ammannia baccifera, Bauhinia variegate, Crataeva religiosa, Hedychium spicatum, Holarrhena antidysenterica, Piper nigrum, Plumbego zeylanica, Psoralea corylifolia and *Saussurea lappa* were screened for tannins, saponins, flavonoids, steroids, cardiac glycosides and alkaloids by Parekh & Chand (2007). Higher concentration of flavonoids was found in *Ammannia baccifera,* cardiac glycosides in *Acorus calamus*, alkaloids in *Ammannia baccifera, Holarrhena antidysenterica, Piper nigrum* and *Psoralea corylifolia* while other phytochemicals were found in minute amount in different plants in different ratio. Parekh & Chand (2007) qualitatively screened twelve medicinal plants (*Abrus precatorius, Euphorbia hirta, Caesalpinia pulcherrima, Cardiospermum halicacabum, Cynodon dactylon, Casuarina equisetifolia, Delonix regia, Euphorbia tirucalli, Ficus benghalensis, Gmelina asiatica, Santalum album and Tecomella undulate*) for the presence of alkaloids, saponins,

steroids, tannins, flavonoids, and cardiac glycosides. High concentration of flavonoids was found in *Casuarina equisetifolia*, while all other phytochemicals were distributed differently in different plants.

Agyare *et al*. (2006) in methanol and petroleum ether extracts of *Nauclea latifolia, Bridelia atroviridis* and *Zanthoxylum gilletii* found saponins, tannins, sterols and alkaloids. Coelho *et al*, (2006) isolated 5,3',4'-trihydroxy-6-C-7-O-β-Dglucopyranosylflavone (a luteolin), 5-hydroxy-7,4'- dimethoxy-6-C-β-D-glucopyranosylflavone (a flavonoid), 5,6,3',4'-tetra-ydroxy-7-O-β-Dglucopyrade and 5,7,4'-trihydroxy-6-C-β-D-glucopyranosylflavone(a isovitexin) from ethanolic extract of *Syngonanthus bisulcatus*. Males *et al*. (2006) stated that different parts of the same plant, same plant collected from different geographic areas and the same part collected at different phase of its growth, *Arbutus unedo* contained different concentrations of flavonoids, rutin, isoquercitrin, hyperosides and quercitrins in all leaves while the fruits contained only chlorogenic acids and isoquercitrin. Farsam *et al*. (2003) studied *Lilium ledebourii* for its phytochemical composition and constituents of essential oils of its flower and oil from its corm. Corm oil contained only fatty acids while flower oils had isopulegol, methylpentacosane, 3-methyltricosane, tricosane, docosane, pentacosane and linalool oxides. Phytochemical constituents detected were alkaloids, flavonoid, saponins.

3. Mineral Elements in plants

Mineral elements are essential part of plant architecture. Some elements are required in large amount by the plants are called as macro-elements (Ca, Na, K, N, P and S) while others, required in minute amount, are called as microelements (Zn, Fe, Cu, Mn, Cr and Ni) or trace elements (Saeed *et al.*, 2010). Elements may be bonded in structural part of an organic molecule in plant or it may be loosely attached and worked as co-factor. The presence of elements and their quantitative composition is important because plants are not only consumed by man and animals as their food but may also be taken in medicinal plants. In both cases the elemental composition of the plant is important due to the fact that their deficiency and excess in food may lead to certain complications. Here are some of the efforts made by different investigators for different plants.

Aftab *et al*. (2011) when analyzed lemon grass, collected from different areas, found that sodium, potassium, calcium, magnesium and silicon were present in high quantity (2.5%, 53.4%, 26.2%, 2%, 10%, respectively). The remaining elements were present below 2% in all samples. Eight species of wild mushrooms collected from 32 types of underlying soil were analyzed by Radulescu *et al*. (2010). They reported high potassium, zinc, iron contents and other macronutrients in fruiting body. Naeem *et al*. (2010) worked on the effects of shape, weight and size (allometry) on the elemental composition of *Oncorhynchus mykiss* collected from different hatcheries. They noted that the isometric increased Cd, Mn, Co, Cu and Cr while Pb, Na, Zn, Ca, Fe and Mg allometric decrease with increase in weight. Similarly Pb, Na, Zn and Ca concentration also decreased proportionally with increase with body length while Ni was below the detecting limit. Hence it was noted that body shape form and mobility have profound effect on mineral element concentration. Omari (2010) analyzed 19 mineral elements in ayuverdic medicines and concluded that only K, Ca, Fe, and Sr were present in satisfactory concentration while the rest of the elements were present below 12mg/kg of dry weight. He mentioned that amount of most of element was below the recommended dietary allowance while certain toxic metals were found above the permitted limit.

Jabeen *et al*. (2010) determined the mineral composition of medicinally important ten plants namely: *Convolvulus arvensis*, *Ricinus communis*, *Achyranthes aspera*, *Justicia adhatoda*, *Alternanthera pungens*, *Brassica campestris*, *Cannabis sativa*, *Hordeum vulgare*, *Parthenium hysterophorus* and *Withania somnifera*. The accumulation of major elements such as Mg, Ca, Na, K and Fe were encouraging. However heavy metals were present in higher amount than the international safety standard. Haloi *et al*. (2010) reported the elemental composition of some vegetables, cereals and legume in Indian Assam. He stated that leaves of coriander, amaranthus and sweet gourd contained variable amount of different elements but iron was below the required level. He further elaborates, that selenium was present in all the analyzed plants but absent in pigeon pea. Similarly, cadmium was present in very low concentration in all plants. Various extracts of *Polygonatum verticillatum* in hexane, ethyl acetate and butanol were assessed for macro and microelements (Saeed *et al.,* 2010). Calcium in all fractions ranged between

100-220ppm, sodium 120-560ppm and potassium 2500-3400ppm while it was found that Ni in hexane and ethylacetate fractions (1.8, 2.4ppm) were above the permissible concentration limit (1.5ppm) and Zn in butanol fraction (60ppm) crossed the permissible limit (50ppm).

Zafar *et al.* (2010) observed that *Silybum marianum, Fagonia indica, Argemone Mexicana, Solanum suratense*, and *Cnicus benedictus* had higher concentration of Fe, Mg, Na, K. Ca, Co and Mn while heavy metals like Ni, Li, Cr, Cu, Pb, Cd and Zn were present in low level as per their need. *Withania somnifera*, collected from two geographical regions, contained high amount of N, P, Mg, Zn, Cu and Mn from Karthikre region while those from Sondekole region were rich in Na, K and Ca. Iron was present satisfactorily in both the cases. This shows that habitat and geography have strong effects on nutritional status and medicinal value of a medicinal plant (Krishnamurthy & Sarala, 2010).

Iwalewa *et al*. (2009) reported that stem bark of *Harungana madagascariensis* had Cl, Ca, Mn, K, and Sr between 10.5 to 774.3mg/g while heavy metals were 1.5 to 7.2mg/g. *Tinospora cordifolia, Gymnema sylvester* and *Tricholepis glaberrima*, used for medicinal purpose, were analyzed by Nile and Khobragade (2009) for elemental composition. They concluded that the plants contained sufficient level of Mg, P, K, Zn, N, Ca, Na and Fe to fulfill the nutritional needs of the people. Nenova (2008) grow pea plants in hydroponics under different concentrations of NaCl and Fe-EDTA to find out how different stresses effect the mineral composition of plants. The effects of NaCl alone and in combination with Fe-EDTA were noted on Na, P, K, Mn, Ca, Zn, Fe, Cu and N composition in the roots and shoots of plants. The overall results showed that the individual effect of one stress on the plant health was equal to the combined effect of the two factors at the same level. Hameed *et al*. (2008) reported that *Polygonum plebejum, Rumex nepalensis, Rheum australe, Rumex hastatus, Persicaria maculosa* and *Rumex dentatus* showed variability of Na, K, Mg, Ca, Cl, Fe, Br, Ti, Si, C, S, O, Al and P among plants and plant parts. Extract of *Cassia obvata* in water, methanol, chloroform, ethanol and ethyl acetate contained nine elements including Al, Zn, Mg, S, Ca, P, Cu, Mn and Fe that have some obvious role in treating skin problems (Pirzada *et al.*, 2007).

It was seen that leaves and pods of *Moringa oleifera* collected from different parts of Punjab (Aslam *et al.*, 2005) contained high amount of Na, Ca, K and Mg as compared to Mn, Fe, Zn and Cu. Phosphorus was present in high amount in leaves only. It was also noted that the plant collected from different locations were different in the amount of their mineral contents. Indrayan *et al*. (2005) found high levels of Mn, Cr, Cu, K and Ca in seeds of *Nelumbo nucifera, Embelia ribes* and leaves of *Artocarpus heterophyllus* but less amount of sodium. While seed of *Eugenia jambolana* were rich in Mg but poor in other elements. Different parts (inffloresccences, grains, stems, fruit, styles, seed, roots, leaves, cobs, shaft, flowers and bud) of the ten plants were checked for mineral element (Adeyeye, 2005). Sodium, potassium, calcium and magnesium were present in large amount in buds of *Hibiscus esculentus*. It was followed by *Lycopersicon esculentum*. There was an apparent variation in different parts as well as different plants but the overall observation was that all plant showed index of bioaccumulation from 1-10. Overall elements were present in decreasing order of Mg followed by Na followed by K and then Ca. Akpanyung (2005) reported that bouillon cubes in Nigeria provide fewer amounts of Na, Fe and Zn than the dietary requirements.

Cabala *et al*. (2004) investigated the mineral composition of rhizpshere and the soil at some distance from plant roots and reported that rhizosphere was rich in hydrated sulphates of Ca, Mg, Fe, primary sulphides of Fe, Zn, Pb and amorphous iron oxides while the bulk soil was poor in these minerals. Niekerk *et al.*, 2004 stated differences in concentration of Ca, Mn, Zn, P, Se and Mg were found *Atriplex canescens, Atriplex halimus, Atriplex nummalaria* and *Cassia sturtii* from different sites. The amount of Mn, Mg and Zn found was sufficient for small ruminants but phosphorus was deficient. Overall it was found that Atriplex spp are good at both macro and micro elements concentration. Chowdhury *et al*. (2004) stated that in some non-leafy vegetables, spices and pulsed had sufficient amount of P, k, Na and Ca to meet the human demand but non leafy vegetables were deficient in Cu, Fe and Mg.

4. Toxic Heavy metals accumulation by plants

Heavy metals are a health hazard. They are present all the time in our ecosystem and due to extensive anthrapogenic activities are continuously increasing. They contaminate our food chain and their increasing amount in food chain can lead health havoc. There is an urgent need to clean our environments from these toxic metals to save our generations from physiological disorders. As there is a very close association between animals, plants, soil and water these metals very easily move from one trophic level to another. One way to clean our environment from these poisonous metals is to search plants that selectively absorb heavy metals from soil and are not poisonous to their own physiology (this action of plant is called phytoextraction). A lot of work has been done which is continuing to search plants that can help clean our environments. Some of the efforts are mentioned below.

Deo *et al*. (2011) examined the possible cleaning effect of the plants present in the vicinity of Bolanda coal mine in subtropical region of India. They concluded that most of the plants removed heavy metals like Cu, Cr and Fe, and store them in different concentration in their body parts. Hence extensive plantation of *Trema orientalis*, *Haldina cordifolia*, *Diospyrous melanoxylon*, *Ixora arborea, Phyllanthus reticulatus* and *Catharanthus roseus* might help protect the area from spread on contaminant metals.

Aisien *et al*. (2010) worked on *Eichhornia crassipes* to check its capability of removing heavy metals from soil. They culture the plant in bore-hole water and dissolved 5mg of Zn, Cd and Pb in the medium. They reported that the metals accumulated heavily in the roots with concentration of 4870mg/kg for Zn, 4150mg/kg for Pb and 710mg/kg for Cd. The accumulatiing ability was fast during the first two weeks and then declined with time due to saturation. This shows the water hyacinth is a good bioaccumulator. To see the impact of altitude on the metal uptake level of plants, Yildiz *et al*. (2010) collected 29 plants sample from an altitude of 1000m and 1600 meter. An overall increase of concentration of Zn, Pb and Mn was noted with increasing altitude while other metals reduced with increasing altitude.

Yap *et al*. (2009) investigated the accumulation of heavy metals (Cu, Cr, Cu, Fe, Mn, Pb and Zn) accumulation in leaves, roots, stem, grain, husks and root zone of paddy

plants. The most accumulated metal in rice leaves, husks and shoots was Mn while predominant metal in the root and grain was Fe. The soil in the root zone was found rich in Mn but the end results showed that accumulated level was not above permissible limit. John *et al*. (2009) while studing the detrimental effects of Cd and Pb in sensitive plant *Brassica juncea* noted decreased chlorophyll, protein, amino acid proline contents and decline in the overall growth of plant. It was observed that Cd was more poisonous than Pb. Both Cd and Pb were deposited most in the roots than in shoots.

Szabo *et al*. (2008) investigated the possible hazardous effects of River Tisza in Hungary to the dependent population by studying the Zn, Co, Ni and Cu concentration in the river, soil and plants grown in the area irrigated with the river water. The results showed that none of these elements posed any threat to the local population due to its low accumulation in the plants that are consumed by the people. Barthwal *et al*. (2008) checked Cd, Ni, Cr and Pb concentration in different medicinal plants (*Abutilon indicum, Calotropis procera, Euphorbia hirta, Peristrophe bycaliculata,* and *Tinospora cordifolia*) collected from residential area, industrial area and heavy traffic area, and stated that heavy metal accumulate differentially in plants due to their exposure to the concentration gradients and hence care must be taken when using them as a medicines. Khan *et al*. (2008) checked the contamination with heavy metals of medicinal plants. They observed that plants not only accumulate essential heavy metals like Fe, Mn and Zn to a considerable amount but also accumulate non-essential heavy metals like Cr, Pb Cd to a hazardous level.

Chehregani & Malayeri, (2007) working on residential plants including *Euphorbia cheiradenia, Scariola orientals, Centaurea virgata, Gundelia tournefortii* and *Eleagnum angustifolia* of Malayer city reported that polluted area had Cd, Zn, Ni, Cu and Pb and that *Euphorbia cheiradenia* was most efficient plant, which efficiently reduced heavy metals. Damian & Damian (2007) studied the effects of zeolites on the absorbtion potential of plant for four metals, Cd, Pb, Zn and Cu. The experimental plants showed excellent growth due to the prevention of heavy metals by cationic exchange mechamins from entering the plant roots and other tissues. Duruibe *et al*. (2007) reviewed the biochemical effects, quantity and quality of the metals causing toxicity, their sources and

caused of release, their movements in the environments, their toxic effects and the biochemistry of how these metals produces their toxic and poisonous effects in animals and human.

Hajiboland (2005) worked on *Triticum aestivum, Medicago sativa* and *Phaseolus vulgaris* to see uptake of Cd, Cr, Co and Ni from soil under labortory condition. *Phaseolus vulgaris* was the most effective while *Medicago sativa* and *Triticum aestivum* were the least effective in cleaning environment from heavy metals.

Kulbat *et al.* (2003) analyzed Municipal waste water for heavy metals including Pb, Cd, Ni, Cr and Hg concentration. They stated that due to biological waste water treatment the metals concentration reduced to normal levels suggesting biological sludge to be an effective technology in land forming and reclamation. Four major metal pollutants namely Zn, Hg, Cd and Pb in Lake Manazala, were monitored in the presence of eight wild plants (*Arthrocnemum macrostachyum, Juncus rigidus, triplex portulacoides, Typha domingensis, Zygophyllum album, Cyperus laevigatus, Phraites austral* and *Salsola sp.*), called as passive bio-monitors and after the introduction of two new bio-accumulator plants called as active bio-monitors (*Raphanus sativa* and *Trifolium alexandrinum*) by Ramadan (2003). It was reported that mercury was present in the lake in a highly toxic level and was a threat for biota, followed in toxicity by Zn, Pb and then Cd. It was noted that the monitoring metals accumulated in highest concentration in active bio-monitors (2.5 to 5times than in passive bio-monitors) followed by sediments (two times higher than the passive bio-monitors) and was least present in the passive bio-monitors. Higher concentrations were observed in roots than in the shoot system while a tendency of co-accumulation between certain pair of elements was also observed as may be in roots or stem or leaves. Prasad *et al*. (2003) reviewed and highlighted the importance of hyper-accumulator plants for cleaning the polluted and metal contaminated ecosystem and have mentioned of 400 metal accumulator plants belonging to eleven important angiosperm families.

Kozaneck *et al*. (2002) discovered that Mn had high concentration in *Cladonia clavatum, Dryopteris filix-mas, Convalaria maialis* and *Vaccinium myrtillus* than Fe. Iron concentration was high in mosses and lichens as compared to Mn. However the

overall concentration of other heavy metals including Zn, Cu, Cd, Ni, Cr and Pb was found with little or no variation as compared to non-polluted areas. Buszewski *et al*. (2002) collected *Pinus silvestris, Festuca pratensis* and *Pleurozium schreberi* during march-april and then in October-November to see for the seasonal effect on the uptake of macronutrients (Fe, Ca and Mg) and trace elements. Maximum differences were observed between the highest and lowest value of Zn (119 to 33mg/kg in autumn and spring respectively). However, overall results showed that heavy metals were in maximum concentration in spring than in autumn and in heavy traffic areas than in residential area. All the values noted were within normal range.

Cladosporium cladosporioides was grown in aqueous extracts from *Vitis vinifera* and *Nordostachys jatamansi* by Pethkar *et al*. (2001). It was found that the fungal species efficiently absorbed Pb and Cd from extract without disturbing other components in the extract. Memon *et al*. (2001) reviewd the difficulties in cleaning environments from pollutants using traditional methods, advantages of bioremediation (removal of heavy metal using plants), the use of genetically modified plants for this purpose, importance of the technology, possibilities of its application and the mechanism how the plants accumulate and detoxify the heavy metals from environment.

Porebska & Ostrowska (1999) analyzed wild plants for heavy metals and reported the highest concentration in *Lactuca serriola, Chenopodium album, Artemisia vulgaris* and *Atriplex nitens*. They calculated from their observation that these plants can remove hundreds of grams of Pb, Cd and almost 20kg of Zn and 2kg of Cu per hectare per year.

5. Antidiabetic potential of plants

Priyanka *et al.* (2010) found that chloroform and alcoholic extract of *Bombax ceiba* (bark), chloroform, alcoholic and aqueus extract of *Grewia asiatica* (leaves), chloroform and alcoholic extracts of fruits of *Luffa acutangula* reduced the BGL significantly in alloxan induced diabetic wister rats as compared to control and glibenclamide. Flower extract of *Sphaeranthus indicus* at 200mg/kg in wister rats was hypoglycemic and proved the traditional claim of Bundelkhand people for its anti-diabetic effect (Jha *et al.* 2010). In a comparative study of traditionally used plant mixture extract at 200mg/kg bw with glibenclamide for 14days, the BGL significantly reduced at

7th and 14th day of the trials which was comparable with glibenamide (Gunjan *et al.* 2010).

Ferula assafoetida extract preserve and protect β-cells and hence maintain BGL as to prevent the diabetic complications (Abu-Zaiton, 2009). Saha (2009) found that hot water extract of *Lagerstromia speciosa* stimulated glucose oxidation through suppression of gluconeogenesis and enhances pentose phosphate shunt pathway, which is apparent due to increase of the activity of Shunt enzyme i.e. G-6-P dehydrognase, enhanced level of glutathione and depression of liver enzyme glucose-6-phosphatase. Sunil *et al.* (2009) found that ethanolic extract of *Posonia alba* had inhibitory effects on α-glucosidase at concentration of 416.7 µg/ml. After 15 days of extract dose BGL, SGPT, SGOT, ALP and cholesterol level decreased while HDL level increased in alloxan induced rats. Singh *et al.* (2009) found that 500gm/kg bw of aqueous extract of *Cynodon dactylon* resulted in 31% fall of BGL in normal and 23% fall in mild diabetic rats. They also noticed that the results obtained were similar to standard drug tolbutamide and in severely diabetic rats if 500mg/kg bw is administered daily for 14days a significant reduction of 59% was achieved in fasting rats. Besides this total cholesterol, LDL and TG levels were also decreased while HDL level (which is cardioprotective) increased in blood. These results shows the hypoglycemic and hypolipidemic potential of the plant.

Euclea undulata, Schkuhria pinnata, Pteronia divaricata and *Elaeodendron transvaalense*, were studied by Deutschlandera *et al.* (2009) for its hypoglycemic activities. *E. undulata* increased glucose uptake up to 162% by hepatic cells with no cytotoxicity while *P. divaricata* and *E. transvaalense* had no inhibitory effect on liver enzymes. The final analysis showed that *E. undulata*, *S. pinnata* and *E. transvaalense* were hypoglycemic if used in diabetes but prolong use be avoided because *S. pinnata* and *E. transvaalense* are toxic to liver. Ghosh *et al.* (2009) tested the aqueous extract from seeds of *Psoralea corylifolia* and *Trigonella foenum-graecum* for the management of streptozotocin induced D. mellitus. The seeds were used separately and mixed in 1:1 ratio for 20days. It was observed that the mixture of the two plants was more potent in normalizing the fasting BGL as compared to individual plant extract.

Selvan *et al.* (2008) found that methanolic extract of *Artanema sesamoides* found effective in controlling blood glucose level. Biomarkers like Insulin, lipid profile, liver glycogen and liver antioxidant were measured and found normal suggesting that the plant is cytoprotective also. Mixture of powdered drugs, with a dose of 400mg/kg, in alloxan induced diabetic rabbits slightly reduced blood glucose level. However, no significant effect was observed on normal rabbits. In the review article Wakdar *et al.* (2008) listed the following plants studied for hypoglycemic activities i.e. *Trigonella foenum-gracecum, Nephoelepsis tuberose, Costus specious, Plantago ovate, Allium sativum, Hemidesmus indicus* and *Allium cepa).* Noor *et al.* (2008) investigated the antidiabetic activity of *Aloe vera* in Streptozotocin induced rats and observed that Islet cells were normalized with *A. vera* extract. Harris *et al.* (2008) while investigating the antidiabetic effects of needle, cone and bark of *Picea glauca* concluded that these plant parts were cytoprotective for PC 12 cells against glucotoxicity and glucose deprivation which is concentration dependent. Habibuddin *et al.* (2008) stated that methanolic extract of leaves of *Securinega virosa* when used on streptozotocin induced diabetic rats intraperitoneally at 100, 300 and 600mg/kg, significantly reduced BGL. *Caralluma sinaica* is chewed in Asir region of Saudi Arabia to decrease BGL. Habibuddin *et al.* (2008) investigated experimentally that administering 100mg/kg bw extraction for 30days to normal and streptozotocin (STZ) treated diabetic rabbits not only brings BGL to normal but also reverses the effects made to liver and kidneys. Glibenclamide was used as standard drug during the experiment and the effects produced in rabbits suggests that CS can be antidiabetic in nature. Antidiabetic efficacy and impacts of extract from neem and bitter-leaf on biochemical indices was checked and compared with chlorpropamide in diabetic rats. Extract alone reduced BGL 29% while in combination with chlorpropamide a reduction of 61% was recorded. Effects on GOT, GPT, total protein, albumin and urea indicated that neem is the best hepatoprotective option. Synergism was observed on combination of neem and bitter-leaf (Ebong *et al.* 2008).

Sokeng *et al.* (2007) investigated the hypoglycemic effects of *Anacardium occidentals* extract in streptozotocin (STZ) induced diabetic rats and concluded that with methanol, n-hexane and dichloromethane extracts at a dose of 175 and 250mg/kg body

weight the blood sugar level decreased by 48%, 45% and 41%, respectively. The glucose level of urine also significantly decreased. Insulin level was increased in diabetic rabbits. It was concluded that the recipe helped in regeneration of β-cells and increased secretion of insulin (Wadood *et al.* 2007). While studying antidiabetic activities of *Equisetum arvense*, Safiyeh *et al.* (2007) observed that extraction with methanol, n-hexan and dichloromethane reduced the BGL significantly after oral administration of 50-250mg/kg daily for 5 weeks in streptozotocin-induced diabetic rats. Hence, the traditional use of *E. arvense* by diabetic patient is supported with this study and further investigation for its active ingredients and mode of action is suggested. Florence *et al.* (2007) tested *Dorstenia picta* for its antidiabetic activities (in normal and streptozotocin induced diabetic rats) by checking its effects on BGL and some biochemical parameters like cholesterol, triglycerides, alanine and aspartate transaminase level (level decreased with extract), creatinine and total proteins (level not changed with extract dose).

Reddy *et al.* (2006) concluded that chloroform, ethyl acetate and alcoholic extracts of the fruit of *Momordica dioica* with a dose of 200mg/kg was antidiabetic in alloxan induced diabetic rats. *Casearia esculenta* is hypoglycemic in both normal and alloxan induced diabetic rats (Arula *et al.* 2006). It was reported that the ethanolic extract of *Casearia esculenta* reduced the BGL to normal and the effect was comparable to tolbutamide. In their review article Bnouham *et al.* (2006) listed important plants investigated for antidiabetic effects by different authors from 1990 to 2000. The listed plants include 176 species from 84 families having confirmed hypoglycemic effects. These are Leguminoseae (11 sp), Lamiaceae (7 sp), Liliaceae (8 sp), Cucurbitaceae (7 sp), Asteraceae (6 sp), Moraceae (6 sp), Rosaceae (6 sp), Euphorbiaceae (5 sp) and Araliaceae (5 sp). The most studied species are: *Citrullus colocynthis, Opuntia streptacantha (*Cactaceae*), Trigonella foenum greacum (*Leguminosea*), Momordica charantia (*Cucurbitaceae*), Ficus bengalensis (*Moraceae*), Polygala senega (*Polygalaceae*), and Gymnema sylvestre (*Asclepiadaceae*).* Kim *et al.* (2006) worked on *Chrysanthemum coronarium, Dioscorea batatas, Morus alba* and *Citrus unshiu*, to see their hypoglycemic or antihyperlipidimic effect. A 100mg/kg bw extract of these plants were given to alloxan treated 36rats for 7days. It was seen that BGL was reduced with

C.coronarium and *M. alba* equivalent to glibenclamide while *C.unshiu* was antilipidemic. *M.alba* reduced triglyceride level of blood.

6. Antilipidemic potential of plants

Obesity is one of the main public health problems in developed countries. Risk factors associated with obesity are development of major chronic diseases, including cardiovascular disease, diabetes and cancer. This is the most common nutrition disorders in the United States (Moreno *et al*., 2003). Some of the efforts to find solution for obesity using plants are discussed here.

Khursheed *et al.* (2010) concluded that Gemmo extract were more efficient in maintaining level of total cholesterol, HDL, LDL and triglycerides in isoproterenol induced myocardial infarction as compared to native plant treated. Gaamoussi *et al.* (2010) investigated the antilipidemic activity in aqueous extract of *Chamaerops humilis* in Meriones shawi rats by measuring plasma glucose, cholesterol, triglycerides and body weight. There was slight decreased in body weight and significant decrease in plasma cholesterol and triglycerides in experimental group as compared to the group given standard drug tourine.

Mansour *et al.* (2009) after investigating the anti-hepatotoxic effect of polyoxyethylenated cholesterol in rats fed with high fat food, concluded that PEO-Chol help normalize liver tissue and also ameliorate plasma glucose and lipid profile. Baeshin *et al.* (2009) concluded that *Rhazya stricta* extract help reduced the TGs, LDL, TC, uric acids and creatinine but increased HDL concentration without affecting liver enzyme activity or kidney functions. Aqueous extract from *Nasturtium officinale* had no effect on plasma lipid level in streptozotocin induced diabetic rats (Shahrokhi *et al*. 2009).

Aloe vera leaf gel extract, at a dose of 300 mg/kg bodyweight per day was antihyperlipidaemic in Streptozotocin induced diabetic rate (Rajasekaran *et al*. 2006). Other factors like Blood glucose, SGPT, SGOT and insulin were also improved to normalcy. Crude polysaccharide extracts (crude LBP), and purified polysaccharide fractions (LBP-X) from *Lycium barbarum* when used in hyperlipidemic rabbits to investigate the effects on serum lipid parameters (total cholesterol, TG, HDL and LDL)

and BGL resulted in significant decrease for glucose but no effect on lipid (Luoa *et al*., 2004).

Moreno *et al.* (2003) while testing the ethanolic extract of *Arachis hypogaea* shells for antilipidemic effects, observed inhibitory effects on pancreatic lipase, lipoprotein lipase and lipolysis, and also noted the effects on body weight gain, liver size and excretion of lipids in feces with high-fat diet in Wistar rats. They found inhibitory effect on lipases, reduced triglyceride contents in liver and increased lipid excretion in feces. Effraim *et al.* (2000) while testing antilipidemic effect of cruide garlic extract alone and in combination with nicotine concluded that garlic alone is more effective than combination in lowering serum lipid level.

7. Hepatoprotective potential of plants

Liver is a highly specialized organ of the body where hundreds of metabolic, storage, secretory and excretory functions is performed daily. A healthy liver mean a healthy individual but liver is always at risk from toxic effects of various drugs, pathogenic bacteria and viruses. To help protect liver from these damages scientists are searching medicines that might help protect liver from damages.

Based on biochemical parameters like SGOT and ALP, Shyamal *et al.* (2010) found that *Ixora coccinea, Rhinacanthus nasuta* and *Spilanthes ciliate* were significant in the protection of liver if treated before the AFB1 induced damage. Based on the positive impact on ALT, AST, AKP and serum bilirubin, *Carica papaya* extract was found hepatoprotective in carbon tetrachloride induced rats (Sadeque & Begum, 2010). Takate *et al.* (2010) while comparing the effects of *Launaea intybacea* extract and silymarin in paracetamol induced hepatotoxicity in albino rats found that the extracts were effective in bringing the level of ALP, SGPT and SGOT to normal. Ahsan (2009) observed that the methanolic extract of *Bixa orellana, Cajanus cajan, Glycosmis pentaphylla* and *Casuarina equisetifolia* were hepatoprotective while that of *Argemone mexicana*, *Physalis minima* and *Caesalpinia bonduc* were not.

Yousef (2009) studied the effects of herbal extracts from leaves of green tea and sage, seeds of fenugreek and caraway, rhizomes of galangal and ginger, resins of

frankincense and myrrh and bark of cinnamon and cinchona on liver of Wister albino rats. Enzymatic factors like ALT, AST, GGT and non-enzymatic factors like bilirubin, urea, uric acid, creatinine, cholesterol, triglycerides and glucose were measured for 30 days and later on histopathology of liver was also studied. He concluded that animals treated with extracts before the toxin dose, showed improvement of liver tissue as compared to those without extract treatment. Ahsan *et al.*, 2009) found that extracts from *Bixa orellana, Cajanus cajan, Glycosmis pentaphylla* and *Casuarina equisetifolia* at a dose of 500mg/kg bw was moderately hepatoprotective in carbon tetrachloride induced liver damage in Swiss albini rats. While *Argemone mexicana*, *Physalis minima* and *Caesalpinia bonducella* had not showed any positive effect. It was further observed that *B. orellana* was the most effective specie which reduced serum level of AST, ALT and cholesterol by 52.08%, 57.37% and 52.90% respectively. Irshad & Mansi (2009) evaluated the biochemical parameters of diabetic rats with extract from *Urtica pilulifera*. They also considered hematological variables (RBC, TLC and PCV). It was found that in diabetic rats' blood glucose, ESR, ALT, AST, urea and creatinine levels increased while RBC, TLC, PVC, Thyroxine (T4), Triiodothyronine (T3) and Thyroid Stimulating Hormones (TSH) decreased. However treating with extract from *Urtica pilulifera* reversed all these effects. Hepato-protective effects of *Annona squamosa* (custard apple) when studied using diethylnitrosamine (DEN) induced swiss albino mice (Raj *et al*. 2009), showed that levels of SGPT, SGOT, ACP, Alpha Fetoprotein (AFP) and bilirubin increased in DEN treated mice but extract dose decreased it. Hence, the plant extract had the ability to protect and repair hepatocytes. Mohan *et al.* (2009) found that at 500mg/kg bw, *Ficus carica* extract had hepato-protective properties equal to silymarin in rats. Garba *et al.* (2009) found that the aqueous extract of *Kohautia grandiflora* is slightly hepatoprotective in paracetamol induced hepatotoxic rats. Biu *et al.* (2009) observed that ALT, AST, uric acid, urea and creatinine values increased while total proteins and albumin level decreased in chickens when treated with aqueous extract from *Azadirachta indica*.

Saleem *et al*. (2008) investigated the hepatoprotective effects of alcoholic and water extract of *Annona squamosa* in isoniazid and rifampicin induced hepatotoxicity

animal model. The standard drug used was silymarin. After checking for the proper functioning of liver, total bilirubin, total protein, ALT, AST, ALP, γ-GT and histopathological study of hepatocytes, it was concluded that *Annona squamosa* extract failed to reverse hepatic injury but it limits the effect of these drugs. Ahmed *et al.* (2008) found three compounds i.e. secologanin, loganin and swerosoid in *Pterocephalus sanctus* but only swerosoid was effective for anti-hepatotoxic activity. Murugaian *et al*. (2008) tested ethanolic extract of leaves of *Wedelia calendulacea* in carbon tetrachloride induced hepatotoxic rats, by monitoring AST, ALT, ALP, protein and bilirubin levels were monitored at fixed intervals and reported that hepatocytes then functioned normal. Extract from fresh leaves of *Cassia occidentalis* showed toxic effects on liver (Nuhu & Aliyu, 2008) and decreased blood protein but raised ALP, AST and ALP levels. Similarly Obianime & Uche, (2008) stated that methanolic extracts of *Phyllanthus amarus* was found to normalize biochemical parameters like AST, ALT, urea, uric acids, total protein, total cholesterol, ACP and AKP in Guinea pigs.

Palanisamy *et al*. (2007) reported that *Pterocarpus santalinus* extract normalized the blood level of AST, ALT, ALP, total bilirubin, lactate dehydrogenase, total cholesterol, triglycerides, albumin and total protein in D-galactosamine induced liver damage in Wistar albino rats. Gupta & Misra (2006) found in paracetamol induced liver damaged albino rats that extract of *Chamomile recutita* reversed the harmful effects of paracetamol on liver glutathione, Na+K+-ATPase, serum bilirubin, glycogen and thiobarbutiric. Hepatoprotective properties of the 50% aqueous ethanolic extract from flowers of *Nelumbo nucifera* was investigated by Rao (2005) against Sprague dawley rats. He found dose related improvement in liver structure along with normalization of ALT, AST, AKP and serum bilirubin which was comparable with silymarin.

8. Plants effect on bone health

A healthy person needs a balance quantity of calcium and phosphorus which are the main constituents of the bone and teeth. Vitamin D along with age of a person also plays a role in calcium metabolism. Ogbunugafor *et al*. (2010) reported that methanolic extract of *Hymenocardia acida* leaves increased calcium level in blood. Al-Attar (2010)

used extract of *Acalypha wilkesiana* leaves for its effect on calcium level in diabetic mice and concluded that detectable elevation calcium level only after 30days.

Atangwho *et al*. (2009) assessed calcium and phosphorus levels in diabetic rats after administration of *Azadirachta indica* and *Vernonia amygdalina* leaf. The effect of both the plants extracts alone and in combination produced significant decrease in serum phosphorus level but with no change in calcium level. The antiosteoporotic effect of *Morinda officinalis* was studied by Li *et al*. (2009) in ovariectomized rat. Administering ethanolic root extract of *Morinda officinalis* arrested bone resorption effects by increasing mineral contents of bone, enhancing bone mineral density and optimizing the calcium and phosphorus level. This shows that overall effect was not the formation of bone but stopping the bone resorption.

Akhtar *et al*. (2007) measured calcium and phosphorus levels in buffaloes suffering from prepartum vaginal prolapsed living near river and compare to those healthy buffaloes living in irrigated and rainy zones of Pakistan. The findings reported lowered level of calcium and phosphorus, which might be the possible cause of the disease. Ambroszkiewicz *et al*. (2007) investigated the impacts of two different food habits i.e. herbivorous and omnivorous on bone development in children between ages 2 to 10 years. They found that calcium, vitamin D, and 25-hydroxyvitamin D was twofold high in omnivorous than in the serum of herbivorous. Moghadam (2006) evaluated the role of inorganic phosphorus rich diet for 14days in broilers. The results showed high ratio of phosphorus and calcium in bone ash and body weight but did not produced any change in plasma calcium and phosphorus level or their ratio while the overall performance of broiler was improved. Basile *et al*. (2006) investigated the function of vitamin D in relation to calcium level in breast milk, maternal serum, calcium secretion in urine and the level in serum every month for three months. They noted that vitamin D at 4000IU/day was more effective than at 2000IU/day in elevating calcium level in both infant and mother. However calcium level declined in breast milk without any relation with vitamin D in maternal blood.

The calcium and phosphorus level of captive caimans was studied at different age, sex, with different feedings and seasons by Latirostris & Yacare (2006) and it was reported that the level of both Ca and P got reduced with increase in age and in winter while the values in free caimans were high than those of the captive in zoo. Adeniran *et*

al. (2006) administered 100mg/kg bw of sherpa plus 280 pesticide and luxan lindane to rats and observed elevated level of calcium in test animal as compared to control.

Yakubu *et al*. (2005) reported that application of stem extract of *Fadogia agrestis* significantly increased the calcium level in blood of albino rates. Prabhavathi *et al*. (2003) studied the changes in calcium and phosphorus level in workers exposed to radiation from processing uranium. They revealed that both calcium and phosphorus levels increased significantly in the exposed workers compared to the control group. Yuk *et al*. (2002) worked on *Carthamus tinctorius* for its anti-bone resorption properties in overiectomized rat. They observed tyrosine kinase inhibition and reduced level of mRNAs involved in prostaglandin and cyclo-oxygenase synthesis. They further suggested that bone resorption process were controlled by inhibiting the phosphorylation of peptides.

Deka *et al*. (1994) observed that methanolic extract of *Cissus quadrangularis* caused bone healing. A faster initiation of bone healing and decreased level of serum calcium level was noted in treated dog compared to control group. Staessen *et al*. (1991) correlate exposure to environmental cadmium and calcium metabolism in 1987 volunteers and found that as the metal accumulate in the body with environmental exposure the calcium metabolism is disturbed which was seen in increase excretion of calcium in urine and raised activity of alkaline phosphates in serum. This shows that cadmium interfere with calcium, vitamin D and collagen metabolism. Looking for substances having animal ovarian extract like activity on calcium level, Mirvish (1929) reported that oatmeal effects in rabbits of both sexes were pronounced which returned to normal level after 72hours.

9. Plants effects on electrolyte levels

Sodium, potassium, chlorine and bicarbonate are the four major electrolytes in the blood which influence biochemical process in body including acid base and water balance, osmolality, electrical impulses in muscles and control heart beats. They are required in body in certain optimal physiological concentration and any deviation from the normal level leads to severe consequences. Their normal level may be disturbed by certain infections, drugs, disturbance in hormonal level, excessive sweating and by the

diet used. To avoid the harmful effects of abnormal level of electrolytes certain medicines are available but an economic and easy to available option is certainly from the plant sources. Thus concerted search is continued in this aspect, which is presented subsequently.

The effects of Neem plant leaves on body chemistry of New Zealand white rabbits was studied by Ogbuewu *et al*. (2010). Neem leaves was administered to four groups of rabbits on the body weight basis. No significant change was noted for potassium or chloride ions while the minimum sodium level was observed at 5% dose while maximum level was noted at 15% dose. The deleterious effects of malaria on electrolyte profile were investigated by Ebele *et al*. (2010) in 150 tested malaria patients of different age groups. Although a lot of variation was noted in age group 10 to 20 years yet overall the sodium and potassium level was significantly reduced in all age groups with malaria infection. Vittal *et al*. (2010) noted significant decrease in serum Mg, K and phosphates level with application of salbutamol nebulization on blood electrolytes in asthmatic patients. The effects of high salt concentration in food of Wistar rats (both treated and untreated with extracts from *Viscum album*) when tested by Ofem *et al*. (2010) showed high concentration of sodium chloride in the diet resulted in increased sodium and chloride and reduced potassium and carbonates in the blood but the effects were ameliorated with *Viscum album* extaract. Lanje *et al*. (2010) studied 50 healthy female volunteers for Ca, Mg, Na and K levels in menstrual, luteal and follicle phases of cycle and reported that Ca and Na were low in luteal phase, while Mg and K in were low in follicular phases.

Akah *et al*. (2009) investigated that methanolic extaract from leaves of *Vernonia amygdalina* increased blood electrolyte level in diabetic rats. Atangwho *et al*. (2009) studied the effects of *Azadirachta indica* and *Vernonia amygdalina* alone each and in combination on serum electrolytes (Na^+, K^+ and Cl^+) profile in diabetic and non-diabetic rats. No significant change was noted in both diabetic and non-diabetic with *Azadirachta indica* extract while with *Vernonia amygdalina* extract only Na^+ level decreased significantly. However on giving combined formulation, serum level of both Na^+ and K^+ was modulated. Rautaray *et al*. (2008) studied deleterious effects of mannitol therapy on

electrolytes level of 25 patients and reported no disturbance to electrolytes during the treatment. Ikpi *et al*. (2009) while studying the effects of aqueous extract of *Rothmannia longiflora* on blood electrolytes in diabetic rats found that the electrolyte level decreased significantly in diabetic control group but remained unchanged in treated with plant extract. This shows that the extract was helpful in maintaining the electrolytes balance in the blood. Ezekwesili *et al*. (2008) used ethanolic extract of *Acalypha torta* leaves in rabbits for its serum electrolytes without showing any activity.

Prvulovic *et al*. (2007) observed that mixing clinoptilolite in pig's food. Increased weight but little or no change occurred in electrolytes. Oboh *et al*. (2007) fed rabbits with carbohydrate and fats rich diet for eight weeks and analyze the effect on electrolyte profile. There was a significant elevation in serum chloride and sodium level while potassium level was dropped and no effect was noted on bicarbonate level. Ibekwe *et al*. (2007) studied plasma electrolytes balance in Wistar albino rats by using of food preservatives sodium benzoate. They noted detectable affects at 60 to 120mg/kg bw where $Na^+$and K^+ levels decreased while $HCO3^-$and Cl^- was not affected.

The change of serum electrolytes with changed season was monitored by Latirostris & Yacare (2006) in *Caiman latirostris*. Highest serum electrolyte level was noted in summer and the lowest in winter. Prohp *et al*. (2006) tested aqueous extract from extra cotyledonous deposit of *Caesalpina pulcherrima* against New Zealand rabbits on blood electrolyte profile. After 28days the level of all electrolytes was found within normal range. Eteng *et al*. (2006) evaluated that after 30days of vitamin C administration there was no significant change in the overall profile of blood electrolytes of Wistar rats. Ethanolic extract from leaves of *Bougainvillea spectabilis* when used against albino rats (*Rattus novergicus*) by Malomo *et al*. (2006) had no significant role in sodium but potassium concentration was reduced significantly.

Detomidine, used as sedative and analgesic in animals, was check for its safety. Khan *et al*. (2003) studied its effects of on blood electrolyte profile using buffalo calves as subject of study. The drug was given 50µg/kg bw and blood samples collected at 30, 60 and 90minutes interval showed no disturbance of electrolytes with detomidine. Kirschbaum (2003) monitored the effects of bicarbonate dialysate on blood acidosis and

electrolytes and noted a shift from acidosis to alkalosis and some ups and down in K and Cl concentration. In another study Oluwole, (2001) noted that 200mg/kg bw dose of garlic for 30days in rats enhanced the Na and K level in blood.

10. Anti-anemic and Immuno-modulatory effects

Hematology or haematology is the study of blood, blood related diseases, blood related laboratory diagnosis and blood components including hemoglobin (Hb), red blood cell (RBCs), white blood cells (WBCs) and platelets (thrombocyts). The main functions of blood and blood components is the supply of oxygen and nutrients to body tissue, removal of wastes from tissue, clotting and defense of body against pathogenic organisms. Any problem with blood and blood component can hamper these functions and can results in deleterious consequences especially weakened immune system can leads to a number of infections. Scientists are searching of plant based medicines for potentiating immune system. Some of these efforts are given here.

Akah *et al*. (2009) reported that methanolic extract of *Vernonia amygdalina* leaves increased white blood cells count in diabetic rat. Taiwo *et al*. (2009) observed that aqueous extracts of *Xylopia aethiopica* and *Phyllantus amarus* decreased erythrocyte sedimentation rate (ESR), while RBCs, WBCs, PCV, total leukocyte count (TLC) and differential leukocyte count (DLC) increased with both extracts in rats. Hb was increased with *X. aethiopica* but not affected with *P. amarus* and hence both the plants show immune booster acitivity. It was noted by Irshad & Mansi (2009) that ESR increased while RBCs, PCV and TLC decreased with induction of diabetes in rats but all these parameters were reversed to normal value after administering 2.0gms/kg bw dose of methanolic extract from *Urtica pilulifera*. Schwerin *et al*. (2009) studied the immune response with plant extract at molecular level in piglets. Using real time PCR they checked for expression of immune responsive marker like *TLR4* and inducible nitric oxide synthetase at 39^{th} day of extract administration. They found up-regulated expression of markers in ileocolic lymph nodes which clearly indicate an elevated immune response. Iwalewa *et al*. (2009) noted remarkable increase in PCV, RBC count and Hb value animals when treated with stem bark of *Harungana madagascariensis*.

Nwinuka *et al*. (2008) administered aqueous extract of Mango (*Magnifera indica*) to normal albino rats for 14days and reported a positively enhanced value of PCV, RBCs, platelets, leukocytes, lymphocytes and negative impact on Hb and neutrophils was noted. The overall results show that the extract enhanced immune response. Ibekwe *et al*. (2007) investigated the potential adverse effect of sodium benzoate on blood and blood components. They observed that both hemoglobin and WBC level decreased in dose dependent manner. The side effect of Sherpa plus 280 pescitides and luxan lindane on hematological parameters were investigated by Adeniran *et al*. (2006). The results showed significant decreased packed cell volume, hemoglobin, red blood cells and white blood cells as compared to controlled group. Adebayo *et al*. (2005) reported that *Bougainvillea spectabilis* ethanolic extract after seven days administration of 200mg/kg bw decreased PCV, Hb and RBCs but did not produced any effect on mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV) and platelets. 50mg and 100mg dosed did not produce any effect. Winkler *et al*. (2005) evaluated the immune-regulatory potential of pumpkin by extracting its seeds in three different solvents and used them against un-stimulated and in-vitro stimulated (mitogen induced) human peripheral mononuclear cells. It was noted that mitogen induced neopterin production and tryptophan degradation was suppressed with extract which shows the immune-regulatory potential of the pumpkin.

Oluwole (2001) stated that 200mg/kg bw of garlic did not produced any detectable effect in rats after 30 days but at 100mg/kg bw dose for the same period of treatment increased the number of RBCs, packed cell volume (PCV) and not only enhanced white blood cells (WBC) count but also improved neutrophil to lymphocyte ratio.

11. Antioxidants in plants

Antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. A variety of free radical scavenging antioxidants exists within the body which many of them are derived from dietary sources like fruits, vegetables and tea.

Patel *et al.* (2010) reported that stem of *Kigelia*, leaf of *Hibiscus, Gemelia* and *Kigelia* had some free radical scavenging activity. Kratchanova *et al*. (2010) working on 25 Bulgarian medicinal plants for their antioxidant activity reported that extraction with acetone (peppermint) not only showed higher polyphenolic contents but also had higher oxygen radical absorbance capacity (ORAC) as compared to water extract.

Adedapo *et al.* (2009) conclued that ethanolic from leaves and stems of *Celtis africana* had comparable antioxidant activity with butylated hydroxytoluene (BHT) as a standard antioxidant used. DPPH radical scavenging activity of the methanolic extract of leaves and flowers of *Lippia alba* was significant as compared to ascorbic acid that showed that the plant is a potential natural source of antioxidants (Ara & Nur, 2009). Zahin *et al.* (2009) studied the antioxidant activity of *Plumbago zeylanica* (Root), *Acorus calamus* (Rhizome), *Hemidesmus indicus* (Stem) and *Holarrhena antidysenterica* (Bark), by ferric thiocyanate (FTC), thiobarbituric acid (TBA) and free radical scavenging (DPPH) methods. They observed that antioxidant activity of these plants differed in order of potency for different methods used. They noticed that the activity was promising, associated with the polyphenolic compounds present in the extract and was in comparable range of standard antioxidants like butylated hydroxyl toluene, L-ascorbic acid and α-tocopherol. *Phoenix dactylifera* and *Citrus aurantifolia* showed high phenolic contents, significant iron chelating and DPPH scavenging activity and the highest protective ability from free radicals as compared to *Loranthus europeas* and *Zingiber officinalis* (Ahmed *et al*., 2009). The antioxidant acitivity and essential oil of *Pelargonium graveolens* (geranium) and *Citrus reticulata* (petitgrain mandarin) were tested. It was noticed that there were 25 components of essential oil in *C. reticulata* and 32 in *P. graveolens* and noticed that radical scavenging activity of *C. reticulate* oil was higher than *P. graveolens*, though the efficacy range of both was near that of standard antioxidants ascorbic acid (Fayed, 2009). *Cosmos caudatus, Polygonum minus, Oenanthe javanica, Centella asiatica* and *Murraya koenigii* were analyzed for its total phenolic contents by Faujan *et al*. (2009) and reported *M. koenigii* to be with highest phenolic yield (38.6mg/100gms) and antioxidant activity hence there was direct corelation of total phenolic contents and antioxidant capacity. Elalla (2009) used Doum fruit extract for its total phenolic contents

and antioxidant activity and reported 0.5 µg/ 3 µg phenolic contents of dry extract and high antioxidant activity.

Ahmed *et al.* (2008) reported that methanolic and aqueous extracts from fruits, shoots and leaves of 21 Jordanian medicinal plants exhibited significant antioxidant activity. When plants were analyzed for their phenolic contents it was found in the range of 4.1 to 365mg/gm of dry weight for methanolic extract and in range of 0.6 to 267mg/gm for aqueous extract. Ayoola *et al.* (2008) worked on *Carica papaya, Magnifera indica, Psidium guajava* and *Vernonia amygdalina* for the presence of phytochemicals and free radical scavenging activity. Phytochemical contents were flavonoids, terpenoids, saponins, tannins and reducing sugars. All plants showed antioxidant activity with *P. guajava* as the most potent. Souri *et al.* (2008) working on different Iranian medicinal plants found that methanolic extracts from *Biebresteinia multifida*, *Polypodium vulgare* and *Allium hirtifolium* had antioxidant activity.

Khalil *et al*. (2007) analyzed eight Egyptian medicinal plants for phenolic compounds and antioxidant activity. He found that salicylic acid was the main constituen amongst the fourteen phenolics. *Sage* showed the highest antioxidant activity, followed by *Dragonhead* and *Plantago* while other the plants showed moderate activity.

Garcinia indica had high antioxidant activity which can be used in cooking, soft drink and as medicinal plant in home-remedies and as a soft drink (Mishra *et al*., 2006). Pourmorad *et al*. (2006) studied plants for their antioxidant activity, phenolic contents and flavanoids. The highest radical scavenging activity large quantity of phenolic contents were recorded for *Mellilotus officinalis* which showed four times more active than butylated hydroxyl toluene (BHT) a synthetic antioxidant. Ahn *et al.* (2006) used natural plant extract (NPE) from Rosemary, Broccoli sprout and Citrus to prevent lipid oxidation of microencapsulated high oleic sunflower oil (MEHS). It was found that NPE prolonged the time of rancidity even when stored for 30 days at 60 ± 1 _C as compared to that without NPE.

Keawpradub *et al.* (2005) found that methanolic extrct of *Arcangelisia flava, Coscinium blumeanum* and chloroform extract of *Coscinium blumeanum* had antioxidant activity due to the presence of triacontanyl caffeate and jatrorrhizine. Gupta *et al.* (2004)

stated that mice treated with methanolic extract from *Bauhinia racemosa* had reduced level of lipid peroxidation and increased level of catalase, superoxide dismutase and glutathione and hence potentiate antioxidant defence system. Similarly, Oke & Hamburger (2002) working on 22 medicinal plants for their antioxidant activity found varied radical scavenging powers.

12. Cytotoxicity and plants

Plants reportedly produce various pharmacological effects due to the presence of secondary metabolites. One such activity is the cytotoxicity against certain cancer cells due to the presence of metabolites like phenylcoumarins and combretastatin A-4 (Yong *et al.*, 2006; Arunachalam *et al.*, 2010). Further search is continued in the hope, that there might be active molecules present in medicinal plants which might effectively overcome cancerous cells proliferation. Some of such investigations are reviewed below.

To see the cytotoxic effect of plant extract Compagnone *et al*. (2010) used MTT cancer assay for oil extract from *Croton matourensis* and *Croton micans* against LoVo, X-17 (colon carcinoma). It was seen that HeLa cancer and control normal cell showed moderate cytotoxic effects only against cancerous cells and not againt control. Molina *et al*. (2010) reported that essential oil from *Origanum majoricum, Origanum compactum* and *Origanum applii* showed cytotoxicity against brine shrimp with LD_{50} value of 10 to 29ppm as compared to aquous extracts which showed LD_{50} equal to water (negative control), clearly indicates the cytotoxicity of the aquous extract. Ethanolic extract from different parts of *Polyalthia longifolia*, *Trachyspermum ammi* and *Elaeocarpus serratus* when used against brine shrimp larvae proved cytotoxic with mild to moderate effect (Sharker & shahid, 2010). *Viscum articulatum* collected from five different plants was extracted with ethanol were used against brine shrimp larvae. All the five extracts showed very potent effects at 1000µg/ml against the brine shrimp larvae (Mutha & Shimp, 2010).

Koffi *et al*. (2009) observed that essential oils from leaves of *Cymbopogon citratus* had pronounced cytotoxic effect at low dose (150µl/ml) as compared to *Cymobopogon nardus* which appeared comparatively less cytotoxic at higher doses (450µl/ml). Shamim *et al*. (2009) conducted a similar study on Iranian medicinal plants stated that methanolic extracts showed cytotoxicity against MCF7 cells. Uddin *et al*.

(2009) extracted 16 plants from Bangladesh with water, dichloromethane, n-hexane and methanol for their cytotoxicity against mouse fibroblasts (normal), humane gastric, colon and breast (cancerous) cells. It was noted that extracts of *Limnophila auriculata* (aqueous), *Hygrophila auriculata*, *Hibiscus tiliaceous* (methanolic) were cytotoxic against breast cancerous cells but were not active against normal mouse fibroblasts. Other plants extract showed low or no activity while *Blumea lacera* (extracted with methanol) was the most potent (IC50 0.01 to 0.08 μg/ml) of all the plants studied. Merghoub *et al*. (2009) reported that extracts from *Ormenis mixta, Retama monosperma* and *Inula viscosa* showed remarkable cytotoxic activity against two cell lines.

Hussain *et al*, (2007) reported significant cytotoxic effect of *Fangonia cretica* against the brine shrimp larvae. Dahab & Afifi (2007) conducted a series of cytotoxic evaluation study on 76 Jordanian plants species belonging to34 families against breast cancer cell line (MCF7) and found that chloroform/ethanolic extract of *Inula graveolens, Salvia dominica, Conyza canadiensis* and *Achillea santolina* were check the growth of cancerous cells. *Inula graveolens* had highest cytotoxicity potential. Aqueous-methanolic extract of *Hibiscus sabdariffa* when tested for cytotoxicity against brine shrimp proved highly effective with LC_{50} value 55.1ppm (Olaleye, 2007). Heo *et al*. (2007) showed that methanolic extracts from *Petasites japonicas* was highly effective against HCT-116 cells while *Erythronium japonicum, Angelica gigas* were intermediate and *Aster scaber* was found the least cytotoxic. Karagoz *et al*. (2007) determined the cytotoxic effects of *Astragalus chrysochlorus* using water, chloroform, ethanol, hexane and ethylacetate extracts against Ver (V) cells in MTT assay. It was noticed that ethanolic extract of stem and roots, hexane extract of stem and chloroform extract of roots and stem were effective cytotoxic at 500μg/ml.

Yong *et al*. (2006) found that combretastatim (a water soluble prodrug) showed 90% inhibition of lung carcinoma cells (A549) at dose of 32μg/ml after 24hrs with cancerous cells.

Keawpradub *et al*. (2005) reported that chloroform extracts of *Arcangelisia flava* and *Fibraurea tinctoria* at dose of 8-12 μg/ml showed some apparent cytotoxic activity against brine shrimp and MCF-7 cell probably due to the presence of berberine alkaloids.

Ee *et al*. (2005) investigated that ananixanthone present in the stem bark of *Mesua daphnifolia* showed cytotoxic activity against human estrogen receptor negative breast cancer (MDA-MB-231), T-lymphoblastic leukemia (CEM-SS cell lines) and cervical carcinoma (HeLa cells). The IC_{50} was found to be 6.7, 1.3 and 4.0μg/ml, respectively.

Pisosterol is a triterpene isolated from *Pisolithus tinctorius* (Basidiomycetes). Pisosterol was highly effective against all tumor cells especially melanoma and leukemia (compared with etoposide and doxorubicin as standard) while there was no cytotoxicity against mouse erythrocyte and sea urchin cell hence the cytotoxic activity was shown only against cancerous cells (Montenegroa *et al.*, 2004).

13. Phytotoxicity

While investigating phytotoxic potential of different parts of *Melia azedarach L,* Lungu *et al*, (2011) observed that aqueous and alcoholic extracts were both inhibitory against seed germination and plantlet growth of *Lactuca sativa L.* Ethyl acetate fraction of the extract of *Zizyphus jujuba* was found moderately phytotoxic against *Lemna minor* L. at 1000 μg/ml, this was investigated by Ahmad *et al*, (2011). Ateeq-ur-Rehman *et al*, (2009) reported that *Thymus serpyllum* L. have no phytotoxicity against *Lemna minor* or radish seeds.

Islam *et al,* (2009) reported that *Oldenlandia diffusa* (Willd.) Roxb significantly inhibited seed germination and root growth of radish seeds. The poor phytotoxic activity of different extracts of *Nepeta juncea* even at higher doses was reported by Hussain *et al*, (2009). In another study Khan *et al*, (2008) used ethanolic extracts of *Trichodesma indicum, Aconitum leave and Sauromatum guttatum* against *Lemna minor* and found them highly effective in inhibiting the plant growth at only 500 pg/ ml concentration.

14. Insecticidal in plants

Insects are always uninvited guests which are often injurious to the human by creating health problems and losses to crops. They are major vectors in spreading diseases amongst the plants, animals and human e.g. malaria and leishminia. The chemical used used for their control creates many environmental and health problems. Hence a safe and effective insecticide is the call of the time and that could be a bio-insecticide produced by living organisms or plants which might be safe to human and

animals but selectively toxic to the insects. Lot of work is in progress in this direction as per following review:

Mallikarjun *et al*. (2010) observed that chloroform, methanol and petroleum ether extracts of *Hemidesmus indicus* and *Swertia chirata* when used against second instar larvae of *Aedes aegypti*, chloroform extract was highly followed by ethanol and petroleum ether extracts. Essential oil from *Ageratum conyzoides, Citrus aurantifolia* and *Melaleuca quinquenervia* when used by fumigation to check its insecticidal potential on *Callosobruchus maculates* (cowpea weevil) showed that *Melaleuca quinquenervia* was more effective than *Ageratum conyzoides* and *Citrus aurantifolia* (Aboua *et al*., 2010). Ashouri & Shyesteh (2010) tested the insecticidal potency of seed powder from *Capsicum annuum* (black pepper) and fruit powder from *Piper nigrum* (red pepper). Both powder were mixed in five different ratio with 20gms wheat grains and was infested with 20adult *Rhyzopertha dominica* and 20 adult *Sitophilus granaries* for 14days and continue to be checked for 36days (after removing the insects for any hatching). Black pepper showed complete mortality to both insects at 14 days while red pepper was not 100% insecticidal. Both plants prevent hatching and development into F1 progeny of both the insects. Sujatha (2010) stated that the root extracts of *Vetiveria zizanioides* in four solvent when tested on *Tribolium castaneum,* found that petroleum ether extract was highly active against both larvae and adult while methanol extract was the least active against larvae and acetone extract the least active against adults insects, other extracts showed intermediate activity.

Vinayaka *et al*. (2009) observed dose dependant mortality of Aedes aegypti larvae by application of methanolic extract of *Abrus pulchellus*, with 100% mortality at 30mg/ml. Methanolic extracts of *Calotropis gigantean* were used against inster larvae of *Tribolium castaneum* by Alam *et al*. (2009) using residual film toxicity method. They reported that chloroform and petroleum ether extracts were insecticidal especially crude methanolic extracts that showed highest activity with very low LD_{50}. Furthermore filter paper repellency test of all the extracts was effective against larvae of *Tribolium castaneum.* Nikkon *et al*. (2009) concluded that the chloroform extract from flower *Tagetes erecta* was highly toxic to *Tribolium castaneum* larvae and adults with LC_{50} of

65.93µg/cm2 while petroleum ether and ethanol extract was not much effective. Muratoglu *et al*. (2009) tested bacterial flora from the body of *Leptinotarsa decemlineata* (a potato beetle) *Leclercia adecarboxylata* an important isolate showed 100% insecticidal effect against the beetle. Sarmah *et al*. (2009) investigated the ovicidal and acaricidal activity of aqueous extracts of *Clerodendron infortunatum, Acorus calamus, Polygonum hydropiper* and *Xanthium strumarium* on the *Oligonychus coffeae* (tea red spider mite) and on mortality and feeding activity of *Stethorus gilvifrons* (a predator of red spider mite). *Xanthium strumarium* produced 87% ovicidal activity, *Acorus calamus* 71%, *Polygonum hydropiper* 31% and *Clerodendron infortunatum* 21%. Acricidal activity was only noted with *Clerodendron infortunatum* and *Xanthium strumarium*. More than 50% in-vitro mortality for red spider mite was observed for all four extracts at concentration of 10%. No mortality or anti-feeding affect was noted for *Stethorus gilvifrons*.

Silva *et al*. (2008) evaluated *Euphorbia antiquorum* latex for insecticidal activity, collected from three different geographical areas and extracted in seven different solvents. They used Potters' spray method, leaf dip method, hand spray method and micro-applicator method against six insects, two predatory coccinellids and one spider. The components of the xylene-latex extract were found effective against the spiders and soft body insects. It was also noted that insecticidal power was different in latex collected from dry, intermediate and wet areas but no seasonal variation in the activity was observed. Haouas *et al*. (2008) tested eight species of *chrysanthemum*, extracted with methanol for the insecticidal activities of their flower and leaves. Leaves extract of *Chrysanthemum macrotum, C. paludosum, C. coronarium, C. grandiflorum, C. myconis, C. trifurcatum,* and *C. fuscatum* when feed to the insect was found insecticidal after three weeks but *C. segetum* showed the least toxicity. Flower extract of only C. *fuscatum* and *C. grandiflorum* was effective when applied topically. Mustafa & Khazraji (2008) evaluated eight plants for insecticidal potential on second instar *Culex pipiens* larvae. *Cleome glaucescens, Quercus infectoria* and *Azadirachta excelsa* was larvaecidal at 200 µg/ml after three days and with LD_{50} between 62.5 to 140µg/ml while *Achillea santolina, Ammi majus* and *Ricinus communis* showed larvaecidal activity after seven days. *Datura stramonium* and *Carum petroselinum* showed no such activity. These experimental

investigations were made by Kumar *et al*. (2008) concluded that fumigation of seeds with 500µg/ml of essential oil from leaves from *Aegle marmelos* prevented *Callosobruchus chinensis*, *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium castaneum* from feeding on seeds, reducing the grain damage and weight loss.

2-tridecanone a monoterpene isolated from *Pilocarpus microphyllus* was used against *Callosobruchus maculates* in non-diluted form and in 1:10, 1:100 and 1:1000 dilution by Braga *et al.* (2007) to check for its insecticidal activity. The seeds exposed to different dilutions of the extract were infested with the insect and then the number of laid eggs, %age of egg hatched, %age of grown adult, average weight of adults, average time for development and number of adults developed was measured. Reduction in laid eggs, eggs hatching and adult development was noted with the extraction treated seeds.

Genetically modified cotton (*Gossypium hirsutum*) with cry1A gene (for Bt-protein) from *Bacillus thuringiensis* was fed to non-susceptible insect armyworm (*Spodoptera exigua*) and the level of Bt-protein was then checked in insect larvae and in their faeces. The secreted Bt-protein was then fed to susceptible insect bollworm (*Helicoverpa armigera*) to check for its insecticidal activity. The results showed that although the quantity of Bt protein decreased in the larval bodies to almost 97.5% but even then the residual Bt protein was active at second trophic level against the susceptible insects larvae (Hong *et al.*, 2006). Khalequzzaman & Sultana (2006) used petroleum ether, ethyl acetate, acetone and methanol extracts from *Annona squamosa* against beetle larvae and adults (*Tribolium castaneum*). Petroleum spirit extract was the most effective (LD_{50} 0.03µg/cm) against the larvae while high toxicity (LD_{50} 58.697µg/cm) against the adult was noted in acetone extract. Hasan *et al*. (2006) tested essential oil from *Acorus calamus* for its toxicity against *Trogaderma granarium*. It was noted that exposure duration was the most important factor in mortality of the insects as only 11.1% mortality was noted on 3rd day but 44.7% at 7th day. Jbilou *et al*. (2006) reported that methanolic extracts of *Raphanus raphanistrum*, *Peganum harmala*, *Aristolochia baetica* and *Ajuga iva* for their insecticidal properties using *Tribolium castaneum* as a target insect. They reported high activity of *Peganum harmala* followed by *Ajuga iva*, *Aristolochia baetica* and *Raphanus raphanistrum*, respectively. Using leaf dip method Rathi &

Gopalakrishnan (2006) discovered the insecticidal property of *Synedrella nodiflora* against *Spodoptera litura*. They found the order of potency of the extract as methanol>benzene>chloroform>petroleum ether>water with LD_{50} ranges from 400 to 600c.

The pediculicide (insecticide) activity against *Pediculus humanus* in essential oil from 54 plants was evaluated in a study conducted by Yang *et al*. (2004). In filter paper contact method eucalyptus, marjoram, pennyroyal and rosemary were more effective while the activity of cade, cardamone, ceylon, clove bud, myrtle, rosewood, and sage was comparable to standard pediculicides 15-phenothrin and pyrethrum. Similarly in fumigation tests the oil from eucalyptus, marjoram, pennyroyal, and rosemary in closed container were highly repulsive than the standard products used.

Kabaru & Gichia (2001) assayed the toxicity of different parts of *Rhizophora mucronata* against *Schistocerca gregaria* (desert locust), 2nd instar larvae of Aedes aegypti and 1st instar larvae of the brine shrimp *Artemia salina*. They reported that bark and pith extract was more toxic to all the three insects, the stem showed intermediate activity while the leaves showed the least.

15. Antibacterial effects in plants

Due to extensive and improper use of antibiotics bacterial pathogens are becoming resistant. There is an urgent need for discovering new antibacterial to treat infections. Scientists are searching different sources for new and novel molecules to be used against resistant bacteria.

Hoskeri *et al.* (2010) reported that crude ethanolic extracts of *Ramalina pacifica* (a a lichen) when used against 20 strains of clinical pathogens i.e. *Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi, Salmonella paratyphi, Escherichia coli* and *Staphylococcus aureus* showed inhibitory activities against *S. aureus* and *E. coli*. The results when compared with Ciprofloxacin as standard drug the zone of inhibition was found significant. Seyydnejad *et al.* (2010) tested ethanolic and methanolic extract of *Callistemon citrinus* and *Albizia lebbeck* to check antibacterial activity against *Streptococcus pyogenes, Bacillus cereus, B. anthracis, Salmonella typhi, Klebsiella pneumonia, Streptococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa* and

Listeria monocytogenes. They observed that these extracts are active against *Bacillus* species but had insignificant activities against other bacteria. Mohamed *et al.* (2010) tested 23 plants for their antibacterial activity against *E. coli, Staphy. aureus* and *Kl. pneumonia*. The highest activity observed was in *Zizyphus spina-christi* against *S. aureus* and *K. pneumonia*.

Secondary metabolites of 35 medicinal plants were identified and used against sensitive and resistant clinical isolates of *Mycobacterium tuberculosis* (Corona *et al.* 2009). It was found that five alkaloid products, 6-methoxydihydrochelerytrine, 6-methoxy-ihydrochelirubine, the flavanone pinostrobin, 1-hydroxy-benzoisochromanquinone and 23-hydroxy-5a-lanosta-7,9,24-triene-3-one were more effective against sensitive strain of *M.tuberulosis* with MIC value of 12.5 µg/ml, while peracetylstrictosidine lactam and the quinone aloe-emodin were less active. Liriodenine was the most effective with MIC of 6.256.25 µg/ml. Methanol and ethanol extracts of *Mentha longifolia* when tested against *E.coli, Staph. aureus, B. cereus, P. aeroginosa* and *B. subtilis* showed that ethanolic extract had antibacterial activities due to the presence of some flavonoids present in the plants (Akroun *et al.* 2009). Arunkumar *et al.* (2009) reported the affectivity *Aloe vera* against *E. coli* and *K. pneumonia*. Oil from *Lippia moldenke* displayed no antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus* and *E. coli* (Owolabi *et al.* 2009). Gende *et al.* (2009) investigated *Cinnamomum zeylanicum* essential oil for its antibacterial effects against *Paenbacillus larvae* (the major cause of honey bee larvae infection) and found that the extract is bacteriostatic at 25-100 µg/ml and bactericidal at 125-250 µg/ml for all strain. Ahmad *et al.* (2009) observed that ethanolic extract of *Ballota limbata* had antibacterial activities against five bacterial species i.e. methacillin resistance *Staph. aureus* (MRSA), *Enterococcus faecalis* (VRE), *Staph. epidermidis*, and *Staph. saprophyticus* but had no activities against fourteen bacterial types of *Providencia rottgeri, Klebsiella pneumonia, K. terrigena, Escherichia coli, Morganella morganii, Kluyvera spp*, and *Enterobacter cloacae*.

Roopashree *et al.* (2008) reported that often extracts of Cassia *tora, Calendula officinalis* and *Momordica charantia* are used for treating different ailments when tested against different bacterial species were found to be more active against bacterial strains

especially *Staphylococcus aureus*. *Chitosan* which is a natural polysaccharide was modified by treating with parabenzoquinone and ethylene di-amine. The modified *Chitosan* was used by (Eldin *et al.* 2008) against four bacterial strains i.e. *E. coli, P. aeruginosa, B. cereus* and *S. aureus*. Modified form was observed more effective than non-modified *chitosan* polysaccharide.

Kotan *et al.* (2007) noted that oil from *Thymus canoviridis, Satureja hortensis, Melissa officinalis inodora, Helichrysum plicatum, Thymus haussknechtii, Thymus sipyleus* and *Thymus sipyleus rosulans* was most effective against *Xaqnthomonas axonopodis* (which is responsible for spot disease on pepper and tomato) as compared to streptocycline used as standard. Guessan *et al.* (2007) studied *Thonningia sanguine* against two sensitive and two extended spectrum β-lactamase (ESBL) producing species of *Escherichia coli* and *Klebsiella pneumonia* and concluded that growth of all the strains were inhibited with extract. Ushimaru *et al.* (2007) found that methanolic extract of *Caryophyllus aromaticus* was more effective against *Staph. aureus* but less against *Escherichia coli, Salmonella typhimurium* and *Enterococcus sp*. Parekh & Chanda (2007) worked on the antibacterial activities of some selected medicinal plants. They observed that methanolic extracts of *Abrus precatorius, Cardiospermum helicacabum* and *Gmelina asiatica* had no activity against *Bacillus cereus, Staphylococcus aureus, Enterobacter aerogenes, Escherichia coli* and *Klebsiella pneumonia* while *Caesalpinia pulchcherrima* was the most active antibacterial plant.

Doughar (2006) suggested that acetone and ethanolic extract of *Tamarindus indica* could be a potential source of antibiotics against both gram positive and gram negative bacteria including *Salmonella paratyphi, Bacillus subtilis* and *Salmonella typhi*. *Lentinula edodes* was grown in 14 different types of culture media by Hassegawa *et al.* (2005). Each was crushed and cell-free filtrate was obtained. All these filtrate was then used against *Bacillus subtilis* to see if the changed growth media has any effect on the biochemistry of plants. The results showed that filtrate from *L. edodes* grown rice bran supplement and vermiculite or molasses was effective against *B. subtilis*. Cvetni & Vladimir (2004) investigated grapefruit, seeds and pulp for antibacterial activities by

using it against 20 bacterial strains and found that only *Salmonella enteritidis* was sensitive at low concentration while rest had some effects at higher concentration only.

Janovska *et al.* (2003) tested 10 different medicinal plants against *Bacillus cereus, E. coli, Staph. aureus, P. aeruginosa* and *Candida albican*. Of them, 5 plants were active against one or more organisms. *Chelidonium majus, Sanguisorba officinalis* and *Tussilago farfara* was found most active against bacteria. Mahesh *et al.* (2008) worked on the antibacterial effects of roots and leaf extracts of *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifer* and *Ziziphus mauritiana*. It was observed that leaves of *A.niltica* and *S.cordifolia*, roots of *Z.mauritiana* showed antibacterial effects. In case of *S. cordifolia* both roots and leaves were active against bacterial strains. Sechi *et al.* (2001) found that *Mycobacterium* was comparatively more susceptible to oil from sunflower (Oleozon) than *Staphylococci, Streptococci, Enterococci, Pseudomonas* and *Escherichia*. They also suggested some clinical trials for comparison with other antimicrobial agents.

Dahot (1998) obtained three protein fractions from leaves of *Moringa oleifera* and used a small protein/peptide against *E. coli, Kl. Aerogenes, Kl. Pneumonia, Staph. aureus* and *B. subtilis*. He noticed that all the three fractions were active against *E. coli, S. aureus* and *B. subtilis* but non against *Klebsiella* species.

16. Antifungal effects in plants

As most of the antifungal agents are toxic to the human and animals health therefore need for less toxic and more effective antifungal plants is always demanded. Important species that are of concern to men are yeasts, molds and dermatophytes.

Cymbopogon citratus, Lantana camara, Nerium oleander, Ocimum basilicum and *Olea europaea* were extracted with methanol, water, diethylether, ethylacetate, n-butanol and chloroform. The extracts were used against *Microsporum canis, M. gypseum, Trychophyton mentagrophytes, T. verrucosum* and *Epidermatophyton floccosum* (Bokhari, 2009). Methanolic extract of *C. citratus, L. camara* and *N. oleander* restricted the growth of *T. rubrum* (85-90%) followed by ethyl acetate extract (80-85%) while aqueous extract inhibit the growth by least (32-77). Of the fungal species *T. rubrum* was the most susceptible followed by *M. canis* then *M. gypseum* and the least *M.*

mentagrophyte. Hadizadeh *et al.* (2009) reported the antifungal effects of some food grade essential oils from medicinal plants including *Urtica dioica*, *Thymus vulgaris*, *Eucalyp spp*., *Ruta graveolens* and *Achillea millefolium* in Iran. Only *U. dioica* and *Thyme* exhibited antimycotic activity against *Alternaria alternata*. Chloroform and aqueous extract of *Cressa cretica* was highly effective against many dermatophytes like *Aspergillus niger, A. flavus, Paecilomyces varioti, Microsporum* and *Trichphyton rubrum* (Pirzada *et al.,* 2009). Bobbarala *et al.* (2009) working on 49 plants for their antimycotic activities against *Aspergillus niger* concluded that methanolic extract of 86% plants,had arrested the growth of fungal hyphae while 14% were without activities. The highest activity was seen in *Grewia arborea*.

Bansod *et al.* (2008) used oil from fifteen medicinal plants and used then against *Aspergillus fumigates* and *Aspergillus niger* applying disc diffusion method. MIC was measured by agar dilution method and minimum cidal Concentration (MCC) was determined by broth microdilution method. Oil from *Cymbopogon martini, Eucalyptus globulus* and *Cinnamomum zylenicum* exhibited the highest antifungal activity as compared to control (miconazole nitrate), while *Cymbopogon citratus* had equal activity to that of control. *Zingiber officinale, Eugenia caryophyllata*, *Azadirachta indica, Withania somnifera* and *Mentha spicata* were moderate in action. *Elettaria cardamomum, Allium sativum, Trachyspermum copticum, Ocimum sanctum, Foeniculum vulgare* and *Cuminum cyminum* had weak antifungal activity. Combination of different oil was more antimycotic than single plant oil. Tra (2008) reported that the plant extract in dichloromethane and 80% methanol in water was used against seven strains of dermatophytes namely *Epidermophyton floccosum, Microsporum canis, M. gypseum, M. langeronii, Trichophyton mentagrophytes, T. rubrum, T. soudanense.* It was found that only extract with dichloroethane was active against *Microsporum canis.* Dulger *et al.* (2008) tested the extract obtained from leaves and rootstock of *Saovia tigrina* was tested against species of *Candida*, *Aspergillus falvus, Penicillium frequentans, Botrytis cinerea*, *Geotrichum candidum*, *Fusarium oxysporum* and *alternaria alternara*. It was seen that these extracts were effective against all fungi especially *C. albican, C. neoformans* and *B.*

cinarea. Essential oils from flowers of *Canlendula officinalis* were effective against 23 clinical fungi strains (Gazim *et al*, 2008).

Acevedo *et al.* (2007) investigated the antifungal activity of protein extract from *Amaranthus hypochondriacus* seeds on *Alternaria alternata, Fusarium solani, Candida albican, Fusarium oxysporum, Trichoderma sp.* and *Aspergillus srchraceus*. A 15mg/ml dilution of extract was inhibitory against all species. The highest efficacy "using poisoned agar method" was found against *F. solani* while the least affected species was *F. oxysporum. Aspergillus schraceus* and *C. Albican* was tested by microspectrophotometery because they don't grow in radial manner. Using microspecrphotometry method *F. solani* and *A. alternata* was not used because they don't produce spores and here the highly susceptible species here was *C. albican* and least was *F. oxysporum*. Satish *et al.* (2007) used aqueous extract of fifty-two plants against eight species of *Apergillus*. *Acacia nilotica, Achras zapota, Datura stramonium, Emblica officinalis, Eucalyptus globules, Lawsonia inermis, Mimusops elengi, Peltophorum pterocarpum, Polyalthia longifolia, Prosopis juliflora, Punica granatum* and *Sygigium cumini* revealed some activities against one or other *Aspergillus* species used for test. As *A. flavus* was more sensitive than other fungal species different solvents were used for extraction and used against this species, of all the extracts the methanolic extract was more effective. Extract from *Luffa operculata,* and *Peltophorum.pterocarpum* with hexane, dichloromethane and ethylacetate were tested against *C. albican* using nystatin as standard drug and solvent as control in disc duffusion, well diffusion, pour plate and streak plate methods. The results showed antimycotic activity of the three plants tested at 30-180mg/10ml of agar (Mohamed & Gomes, 2007). Thirty fungal isolates of Pestalotiopsis microspora and two isolates of *Bartalinia robillardoides* were collected from roots, bark and twigs of *Azadirachta indica, Holarrhena antidysenterica, Terminalia arjuna* and *T. chebula* (Tejesvi *et al,* 2007). These isolates, extracted with ethylacetate, were used against *Alternaria carthami, Fusarium oxysporum, F. verticilloides Macrophomina phaseolina, Phoma sorghina* and *Sclerotinia sclerotiorum*. Extract from *Terminalia arjuna* was more antimycotic than those from other plants with inhibition zone of 4-25mm diameter. Hence further study is recommended. Khan *et al.*

(2007) concluded that *Rhazya stricta* extract in pet-ether, chloroform, carbontetrachloride and methanol were checked for antifungal activities against *Trichophyton longifusis*, *Aspergillus flavus*, *Candida albicans, Microsporum canis* and *Fusarium solanibut* only chloroform and methanolic extract is active against fungal growth.

Using ozone generator, Ozonized olive oil were used against pathogenic fungi (*Aspergillus fumigates*, *Candida albican*, *Epidermatophyton floccosum*, *Microsporum canis* and *Trichophyton rubrum*). MIC was found active to range from 0.53-2.0mg/ml for all species. Reduction in amylase, Keratinase and urease enzymes activities in all fungal species were noted (Geweely, 2006). Ethanolic extract of seeds and leaves of *Moringa oleifera* had antimycotic activity against *Trichophyton rubrum*, *T. mentagrophytes*, *Epidermophyton Xoccosum*, and *Microsporum canis* and found effective. GC analysis showed 44 compounds in the extract (Chuang *et al.*, 2006).

Hemly *et al.* (2005) collected 158 yeast isolates of 23 species from chicken burger in different markets of Tanta city and were tested against Cinnamon, Cove and Thyme extracts. The extract showed potent antifungal activity against *C. albican*, *D. hansinij* and *S.cerevisiae* at 20% concentration while at 10% concentration the inhibitory activity was moderate. Garlic extract had lesser activity at this concentration. Winkelhausen *et al.* (2005) tested phenolic compounds extracted from olive pomace to check whether they are able to inhibit growth of fungal species *Alternaria solani, Botrytis cinerea* and *Fusarium culmorum*. The end results showed that the fungal growth was arrested by these compounds. Ahmed & AbdelGaleil (2005) found two types of sesquiterpene lactones, costunolide and parthenolide in the methanolic extract from stem bark of *Magnolia grandiflora*. They used both these sesquiterpene against plants pathogens *Alternaria alternata, Helminthosporium spp, Nigrospora spp, Fusarium oxysporum, Fusarium culmorium* and *Rhizocotonia solani*. Costtunolide was strong against *Nigrospora* spp, *R. solani* and *Helminthosporium* spp. while Parthinolide showed good antifungal action against *A. alternata* and *F. culmorium*.The author suggest that these lactone rings can be used for development of a new class of antifungal. Ramisz *et al.* (2005) reported that solution of Chitosan in acetic acid inhibited the growth of pathogenic fungi namely; *Candida albicans, Trichophyton mentagrophytes* and *Microsporum canis. Erigeron*

floribundus is a medicinal plant used in West Africa for the treatment of skin disorders. Of the eighteen compounds isolated from *Petiveria alliacea* mentioned by Kima (2005) only thiosulfinates, trisulfides and benzylsulfinic acid were more active.

Broth microdilution, disk diffusion method and checkerboard microtitre assay was applied. Synergism was seen with Ketoconazole. Crude extract of *Tamarix dioica* was antimycotic against *A. flavis*, *M. canis* and *F. solani* was reported by Khan *et al.* (2004). Vermicompost aqueous extract was found to exhibit activity against some of phytopathogenic fungal species and some saprophytes i.e. *Alternaria solani*, *A. brassicae*, *A. alternata*, *Curvularia penneseti*, *Curvularia*. sp., *C. maculans*, *C. palliscens*, *Helminthosporium speciferum*, *H. penniseti*. The extract inhibited spore germination and development of powdery mildews on balsam (Singh, 2003). Hammer *et al.* (2003) reported that the antifungal activity in *Melaleuca alternifolia* is mostly due to terpinen-4-ol, a-terpineol, linalool, a-pinene, b-penene and 1, 8-cineole. Essential oil extracted from *Agastache rugosa* were used alone and with ketoconazole against *Blastoschizomyces capitatus* by Shin *et al.* (2002). Manohar *et al.* (2001) stated that volatile aromatic oils from *Origanum* and *carvacrol* contain phenolic derivatives when used against *Candida albican* with daily administration of 8.6mg in 100 µl of olive oil/kg body weight for 30days in infected mice resulted in 80% survival rate.

17. Antitumor effects in plants

Agrobacterium tumefacien is the causing bacteria of crown gall (plant tumour). The bacterium produces the tumour with help of plasmid containing the necessary information for converting normal cell to tumorous cell. The bacteria can be used experimentally for initiating tumour in presence of pure compounds and plant extract to check their ability of preventing abnormal cell proliferation. The following are some of these efforts done in this respect.

Mazid *et al*. (2011) investigated *Polygonum barbatum* and *Polygonum stagninum* for their antitumor potential using potato disk assay. They reported that petroleum ether extract of *Polygonum barbatum* and n-hexane and ethyl acetate extract of *Polygonum stagninum* at 180 µg/disc were able to arrest tumor growth while methanolic extracts of both plants had little or no activity with IC_{50} value >400µg/disc. In the end *Polygonum*

stagninum was found more effective than *Polygonum barbatum*. Mon *et al*. (2011) reported antitumor activity of *Ardisia japonica*, *Ageartum conyzoides* and *Cocculus hirsutus* using carrot discs instead of potato discs infected with *Agrobacterium tumefaciens*. They noticed inhibition of gall development (after three weeks of incubation) with aqueous leaves extract for all the three plants but the highest activity was observed with *Ardisia japonica*. Hosseinzadeh *et al*. (2011) observed that that the aqueous and methanolic extract of *Allium sativum* did not inhibit growth of *Agrobacterium tumefaciens* but effectively inhibited the ability of bacteria to induce tumor with IC_{50} of 0.92mg/ml and 0.75mg/ml for the two extracts. Maw *et al*. (2011) reported that *Gynura procumbens, Achyranthes aspera* and *Polygenum tomentosum* when tested for antitumor activity found that *Achyranthes aspera* at 100ppm arrested the tumor growth while *Gynura procumbens* and *Polygenum tomentosum* were effective at 1000ppm.

Noudeh *et al*. (2010) tested methanol, petroleum ether and also essential oil extracts from *Heracleum persicum* and *Cinnamomum zeylanicum* for their ability to arrest tumor growth. It was observed that both *Heracleum persicum* and *Cinnamomum zeylanicum* inhibited crown gall induction by 57.16% and 72.90%, respectively. The oil from both plants also exhibited activity against tumor growth with IC_{50} of 2.24 and 1.20mg/ml for *Heracleum persicum* and *Cinnamomum zeylanicum*, respectively.

Methanolic leaf extract from *Oldenlandia diffusa* inhibited *Agrobacterium tumefaciens* induced tumor growth at 100-1000ppm concentration as reported by Islam *et al*. (2009).

Ateeq-ur-Rehman *et al*. (2009) reported that methanolic extract of *Thymus serpyllum* inhibited tumor growth. Kuete *et al*. (2009), extracted stem bark of *Antiaris africana* with ethanol and tested against tumor producing bacteria *Agrobacterium tumifaciens*. They results they reported 83.12% reduction in tumor development. Analysis of the plant showed that methanolic extract contained a compound 3, 39-dimethoxy-49-O-β-d-xylopyronosylellagic acid which might be the possible inhibitor of the gall.

Two synthetic molecules N-(2-(1-benzyl-2-(alkylthio) -1H-imidazole- 5-carboxamido) ethyl)-1-benzyl-2- (alkylthio)-1H-imidazole-5-carboxamides and N-(2-aminoethyl)-1-benzyl-2- (alkylthio)-1H-imidazole-5-carboxamide were evaluated for antitumor activity using potato disk assay. 75.52% and 67.24% tumor inhibition was observed at 1mg/ml for first and second molecule respectively as compared to Vincristine 100% as standard drug at 0.25mg/ml (Hadizadeh *et al.*, 2007). Hussain *et al*. (2007) experimentally found that the extract of *Fagonia cretica* used for treating cancer when used against tumor producing three strains (At6, At10 & At77) of *Agrobacterium tumfaciens* on potato discs inhibited of tumor growth by 77.04%. The extract did not affect normal growth of bacteria.

Ibrahim *et al*. (2005) tested ethanolic extract of 17 algal species for antitumor effects against *Agrobacterium tumifaciens* inducing tumor. Only *Codium tomentosum, Padina pavonia* and *Jiana rubens* exhibited some cytotoxic activity.

CHAPTER 3

MATERIALS AND METHODS

1. Research Plants

After enquiries from herbal therapist (locally called as hakeems) and elders about the medicinal properties of plants, *Euphorbia prostrata* (EP) and *Euphorbia granulata* (EG) were selected for present work.

Collection of plants: Both the plants were frequently available throughout Khyber Pukhtoon Khwa province everywhere. The plants were collected from Peshawar University Campus, from three different habitats; 1. Along footpaths, 2. Along waste water streams sides and 3. from gardens/lawns. The plants were collected during the month of July – August, at reproductive stage mostly in after noon. Whole plant including roots, stem, leaves and inflorescence/seeds were collected by hand picking. Roots were digging out carefully.

Handling and processing: the plants were washed thoroughly with clean tap water followed by rinsing thrice with distilled water. The plant were separated into roots, stem and leaves and also kept as whole plant was also used. The plant materials were then shade dried. Dried plant materials were powdered in blender. Seeds were also collected, dried and stored on paper bags.

Extraction: hundred grams of each of the whole plant materials and 20 gms each of dried roots, stem and leaves were separately soaked in 500ml and 100ml of 70%ethanol respectively. They were kept for one week. On eighth day each sample was filtered through whatman No 2 filter paper. The filtrates were stored in a separate containers and the residue was subjected to soxhlet apparatus for further extraction. Extractions continued till no more green color appeared in the extracting solvents. All the extracts of same parts were then mixed together. They were then evaporated under reduced pressure in rotary evaporator. The semisolid deep brown color extract for each sample was stored in air tight container.

Preparation for activities and analysis

For analytical purpose both powder plant materials and extracts were used. While for bioassay technique only extracts were used.

Preparation of stock solution for activities

Hundred mg / ml of the extracts for both *Euphorbia prostrata* and *Euphorbia granulata* were dissolved in three solvents, i.e. Dimethysulfoxide (DMSO), ethanol and water as stock solution that were stored at room temperature for activities.

2. Nutritional Analysis

1. Detection (Screening) Tests

Before going into detailed analysis for different nutrients the plants parts were screened for the presence of carbohydrates **(**all types), proteins and amino acids. Colors producing chemical reactions were used for detection.

Carbohydrates: 20gms of dry powder of both plants were placed separately in 20ml of water for 72hrs. Thereafter were filtered through whatmann filter paper to check for the presence of carbohydrates.

Following Masood (2006), to 1ml of extract few drops of Molish reagent were added in a test tube. Addition of 0.2ml concentrated sulfuric acid resulted in violet color that indicated the presence of carbohydrates.

Reducing sugar: Masood (2006) method was followed. To 1ml of extract few drops of Benedict's reagents was added in a test tube and heated in boiling water. The appearance of reddish brown coloration indicated the presence of glucose, fructose, galactose, lactose, sucrose and maltose etc. The test for reducing sugar was confirmed following the same author. To 1ml of extract Fehling's reagent (mixture of equal volumes of Fehling A and Fehling B) was added in a test tube. On heating in boiling water the appearance of brick red color confirmed reducing sugars.

Test for monosccharides: the method of Bahuguna (2007) was followed. To 1ml of extract 1ml of Barfoed's reagent was added in a test tube and heated in boiling water for two minutes. The appearance of red color indicated the presence of monosaccharide.

If the monosaccharide are absent then continue heating for almost ten minutes the disaccharides gives red color on prolong heating.

Test for ketose sugar: following Masood (2006), to 1m of extract 5ml of Selwinoff'r reagent was added in a test tube and heated in boiling water. The appearance of red color within 30 seconds indicates fructose and other ketose sugars like dihydroxyacetone, erythrulose, ribulose, xylulose etc.

Test for Aldose sugar: after Masood (2006), 0.1mg of dry ethanolic extract and 2ml Tollen's reagent was added in a test tube. The formation of silver mirror on inner side of test tube indicates aldose (glucose and glyceraldehydes etc) sugar.

It was further confirmed through Masood (2006) method. To 0.5ml of bromine water 1ml of extract was added in a test tube. The discoloration of bromine confirms the presence of aldose sugar.

Tests for Proteins and Amino acids

Test for protein: To 1ml of extract 1ml of conc. HNO_3 was added in a test tube. The appearance of yellow color indicated the presence of proteins (Masood, 2006). It was confirmed using Bahuguna (2007) method. Here 1ml of extract and 1ml of Millon's reagent were mixed in a test tube. The appearances of white color precipitate which turn red with heating confirmed protein.

Test amino acids: after Tiwari (2011), 1ml of extract was added to 1ml of 0.2% Ninhydrine in a test tube. The appearance of violet color indicated the presence of amino acids. The test is not highly specific for amino acids because short stretches of protein also produces the same color on heating with ninhydrine.

2. Quantification of nutrients

Ash, moistures, fibers, fats, proteins and carbohydrates were quantified in whole plant, root, stem, leaves and seeds of both tested plants following Ezeibekwe *et al*., (2009).

Moisture contents

Protocol used by Ezeibekwe (2009) with minor modification was followed using Gravimetric method. Two grams powdered plant of each sample was taken in a clean dry crucible weighed and was kept for 5hrs at $105C^0$ in electric oven. At the end the crucible

with dried sample was weighed again. The experiment was performed in triplicate for each sample. Moisture was calculated using the formula

$$\text{Moisture (\%)} = \left({}^{Wb}/_{Wa}\right) \times 100$$

Ash contents

Protocol used by Hussain *et al*. (2010) with minor modification was followed, using incineration gravimetric method. Two grams of powdered whole plant and parts was taken in a clean dry crucible, weighed and placed in furnace. The temperature was slowly raised to 600^0C and maintained for five hours. The sample was visually checked for complete ashing (white or grayish white). Crucible along with ash was cooled in desiccators and weighed. Three repeatitios were made for each sample. The experiment was performed in triplicate for each sample. %Ash was calculated using the formula

$$\text{Ash (\%)} = \left({}^{Wash}/_{Wpow}\right) \times 100$$

Crude lipids

Using Solvent extraction method, Aberoumand (2009), with minor modification was followed. Two grams of dried and powdered sample was taken into a porous thimble. The mouth of thimble was closed with cotton plug and placed in extraction chamber of soxhlet apparatus. Weight of receiving chamber was noted before the extraction process started. The receiving chamber was half filled with petroleum ether. The heating mental was set at 60^0C (B.P. of petroleum ether). For complete extraction of crude lipids the heating process was continued for 8 hours. The thimble with the plant residues was removed and the solvent were distilled off. Solvent in the reservoir contain crude lipids and so the solvent were evaporated. The reservoir along with the residue was weighted. The experiment was performed in triplicate for each sample.

Crude fibers

Protocol used by Aberoumand (2009) with minor modification was followed, using Weende method. 1.25% H_2SO_4 (by dissolving 7.2ml concent. acids in 1000ml H_2O) and 1.25% NaOH (dissolving 12.5gms pellets in 1000ml H_2O) was prepared in a beaker. Two hundred ml of 1.25% H_2SO_4 was heated to boiling in a 600 ml beaker. The residue of the fats extract (whole plant and parts) was put into it. After 30 minutes of boiling the residue was cooled and filtered through a filter paper. It was washed three

times with boiling distilled water and was returned to the beaker again. It was boiled in the beaker for 30 minutes in 1.25% of NaOH and filtered again. Washed with 50ml boiling distilled water and finally with 25ml of ethanol. Placed in oven for 1hour at 105^0C to dry completely and then cooled in a dessicator. Scratched the residue into a pre-weighed crucible, weighed and was placed in furnace for five hours at 600^0C. Visually checked for complete ashing (white or grayish white) and the crude fiber % contents were calculated as the % weight lost on ashing.

Crude protein and nitrogen contents

Protocol used by Indrayan (2005) with minor modification was followed using Kjeldhal approach (digestion, neutralization and titration approach). Two grams of dried and powdered sample (whole plant and parts) was placed into Kjeldhal digestion flask. To the flask 10ml sulfuric acids and 1gm anhydrous copper sulfate (catalyst) was added to speed up the digestion. Two drops of tributyl citrate added as antifoaming agent and 5gms of K2SO4 was added to lower the boiling point of the mixture. The mixture was heated to boiling on heat block for digestion until a clear solution was obtained. The resultant digested sample was cooled and filtered into a flask. The final volume of the filtrate was made up to 100ml with distilled water. 10 ml from this sample preparation was added to distillation flask. Twenty ml of 45% NaOH solution was added to make it alkaline which changes ammonium ion to ammonia gas. The flask was connected to a trapping flask fit with condenser, containing 50ml of 20% boric acids (H_3BO_3) and methyl red as indicator. The flask was heated to boiling on heating block until all the NH_3 is evaporated completely into the trapping flask (this NH_3 neutralizes some acids in the flask). The remaining H_3BO_3 in the trapping flask is then titrated against the standard base (0.1N NaOH) and the end point is noted with change in the indicator color. The protein contents are calculated first by knowing the %age of nitrogen in the sample using the formula.

$$\text{Nitrogen (\%)} = \left(\frac{Tv \times N.H2SO4 \times Df \times 100}{Wps}\right) \times 100$$

and then by multiplying the value with 6.25.

Crude protein = %age of Kjeldhal nitrogen X 6.25

Where 6.25 is a nitrogen conversion factor.

Carbohydrates (%)

It was determined by Indrayan (2005) method with minor modification. Carbohydrate contents were calculated by subtracting the values of ash, moisture, fibers, fats and proteins from 100 (whole plant and parts). The formula is as given below

$$\%\ \text{carbohydrates} = 100 - (\%\text{protein} + \%\text{fats} + \%\text{ash} + \%\text{moistures} + \%\text{fibers})$$

Nutritive value was calculated as below

$$\text{Nutritive value} = 9 \times \%\text{age of fats} + 4 \times \%\text{age of carbohydrates} + 4 \times \%\text{age of protein}$$

3. Phytochemical analysis

1. Detection (Screening) Tests

Before going for detailed analysis of alkaloids, terpenes, terpenoids, tannins, saponins, glycosides, anthraquinones, phenolics, flavonoids, phlobatannins, anthocyanins, coumarins, steroids the plants parts were screened for their presence. This was done by reacting the extracts with certain reagents and appearance of characteristic color. The presence of these phytochemicals was then confirmed with some other test as well.

Detection of alkaloids

The method of Tiwari (2011) was followed by taking 4gms of the extract to which 4ml of HCl was added in a test tube and 1ml distributed into four separate test tubes and marked.

Mayer's Test: The appearance of yellow color by adding few drops of Mayer's reagent indicated the presence of alkaloids.

Wagner's Test: The appearance of brown/reddish color by addition of few drops of Wagner's reagent confirms the presence of alkaloids.

Dragendroff's Test: The appearance of red color with addition of few drops of Dragendroff's reagent confirmed alkaloids.

Hager's Test: The appearance of yellow colour with addition of few drops of Hager's reagent confirmed alkaloids.

Detection of terpenes

Terpenes are technically only hydrocarbons that were determined by method used by Masood (2006).

Test for diterpenes: (Copper acetate method) 1ml of water extracts of *E. prostrata* and *E. granulata* was treated with few drops of copper acetate solution. The appearance of emerald green color detected diterpenes.

Test for triterpenes

Two methods were used. The first method is called as Libermann-Burchard test, where 1ml of water extracts of *E. prostrata* and *E. granulata* were treated with acetic anhydrides and boiled in a test tube. After cooling, the sample was treated with conc. H_2SO_4 drops wise along the wall of the test tube. The appearance of deep red color at the point of junction represents the presence of triperpenes. The second method is called as Salkowski's test. 1ml of Water extracts of EP and EG was treated with conc. H_2SO_4 drop wise and shakes well. On standing for few mints the appearance of yellow color at lower side of the test tube confirm triterpenes.

Detection of terpenoids

Terpenoids differs from terpene are being oxygenated terpenes. They were detected through Salkowski test. 0.5gms of each of *E. prostrata* and *E. granulata* were mixed with 2ml of chloroform in a test tube. To each sample was added 3ml of conc. H_2SO_4 carefully from the wall of the test tubes. The appearance of reddish brown color at the junction shows the presence of terpenoids (Ghafour *et al*, 2010). For confirmation to 5ml of each plant part extract of both *E. prostrata* and *E. granulata* OR 2ml of chloroform added in a test tube. It was then treated 3ml with conc. H_2SO_4 slowly and gradually along the walls of the test tube. The appearance of reddish brown color confirmed terpenoids (Kanife *et al*, 2012).

Detection of tannins

Tannin is astringent taste natural polyphenolic compound. It was detected using Masood (2006) techniques.

Gelatin test: To 1ml of extract, a mixture of 10%Nacl and 1% gelatin was added dropwse in a test tube. The appearance of white ppt shows the presence of tannins. It was

further confirmed by applying ferric chloride test. To 1ml of extract, 1ml of ferric chloride solution was added in a test tube. The appearance of blue green coloration confirmed the presence of tannins.

Vanillin hydrochloride test: The appearance of purple red coloration with added vanillin hydrochloride to 1ml of extracts drop wise in a test tube confirms the presence of tannins.

Alkaline reagent test: The presence of tannins was further confirmed by adding 0.1N NaOH was added drop wise to 1ml of extracts which gives yellow to red coloration.

Detection of saponins

Tiwari (2011) procedure was followed for detecting saponins. The amphipathic glycosides found as secondary metabolites in plants was detected by using the following 2 tests.

Froth Test: To 5ml stock solution of *E. prostrata* and *E. granulata* 15ml of dist. water added in a graduated cylinder. The samples were vigorously shaken for 15 minutes. The surface of each sample was examined visually. The appearance of 1cm layer of froth on the sample surface showed the presence of saponin. This was confirmed using foam test. To 2ml of water 0.5 gm of each of *E. prostrata* and *E. granulata* extracts were added in test tube and shaken vigorously. The formation of foam in mixture which stayed for 10 minutes confirmed the presence of saponin.

Detection of glycosides

Glycosides were detected following the method of Bahuguna (2007).

Detection test: 1gm of each of the two extracts hydrolyzed with 10ml hydrochloric acids at $80C^0$ in a water bath for 4hrs.

Modified Borntrager's Test: 2ml from the above hydrolysate was treated with was 2 ml of ferric chloride solution in a test tube and heated to boiling in boiling water for 10 minutes. After cooling to room temperature, the samples were mixed with benzene in 1:1 ratio, shaken vigorously and after separating the layers, ammonia was added to the benzene layer. The appearance of rose pink coloration in ammoniacal layer shows the presence of glycosides. Legal's test was used to confirm the presence of glycosides. To 2ml of this hydrolysate 1ml of pyridine was added in a test tube and then sodium

nitroprusside drop wise. Then NaOH was added drop wise to the mixture until the appearance of pink to red coloration which shows the appearance of glycosides.

Detection of anthraquinones

To 6ml of 1%HCl 1gm of the extracts of *E. prostrata* and *E. granulata* was added, dissolved, filtered and shaken vigorously with 5ml of benzene in a test tube. The benzene layer was separated and treated with few drops of 10% NH_4OH. The appearance of pink/violet/red coloration showed that anthraquinone are present (Kumar *et al.*, 2009). The Confirmation was done by adding 10ml of H_2SO_4 to 500mg extract of *E. prostrata* and *E. granulata* in a test tube and heated in boiling water for few mints. After cooling to room temperature the sample was filtered and treated with 5ml chloroform. The chloroform layer was separated and treated with 1ml of ammonia in another test tube and the color change of the sample observed (Ayoola *et al*, 2008)

Detection of Phenolics

Method used by Masood (2006) was followed for phenolic compounds. Shinoda Test or also called as Magnesium Hydrochloride reduction test was applied. To 1ml of stock solution of *E. prostrata* and *E. granulata* few fragments of magnesium ribbon and few drops of conc. HCl was added in a test tube. The appearance of crimson red/pink scarlet or green coloration showed that phenolic compounds are present. Confirmation was done using Zinc-Hydrochloride reduction test. To 1ml of stock solution of *E. prostrata* and *E. granulata* was added a mixture of zinc dust and HCl. The appearance of red coloration within a few minutes confirms the presence of Phenols.

Detection of flavonoids

Ayoola *et al*, (2008) procedure was followed for detecting flavonoids.

Detection Test: 500mg of *E. prostrata* and *E. granulata* extracts were dissolved in 1ml water and added to 5ml of ammonia in a test tube. The production of yellow coloration on addition of 1m conc. H_2SO_4 which disappears on standing showed the presence of flavonoids. It was confirmed dissolving 500mg of *E. prostrata* and *E. granulata* dried extracts in 1ml water and 1%aluminium solution was added drop wise. The appearance of yellow coloration confirms the presence of flavonoids. It was further confirmed by dissolving 500mg of *E. prostrata* and *E. granulata* extracts in 1ml water and 10ml of

ethyl acetate added in a test tube above hot boiling water for three mints. 2ml from the above sample solution was treated with almost 0.5ml 1% aluminium solution. The appearance of yellow coloration confirms the presence of flavonoids.

Detection of phlobatannins

Phlobatannins is a condense tannin. The method used was that of Edeoga *et al*, (2005). 10mg extract of *E. prostrata* and *E. granulata* were dissolved in 2ml of water, mixed and heated with few drops of 1%HCl in a test tube. The appearance of red coloration showed the presence of phlobatannins in the sample.

Detection of anthocyanins

Anthocyanins are glucosides of anthhocyanidins which impart different colors to different parts of plants. Masood (2006) procedure was followed for detecting anthocyanins.

At different pH: To 1ml of aqueous stock solution of *E. prostrata* and *E. granulata* few drops of dil. HCl added in a test tube and wait. Yellow to orange coloration develops which on addition of 1ml of 1M sodium hydroxide (NaOH) turns greenish to violet after some time. On addition of 1ml more NaOH the color changes to brown or orange that showed the presence of anthocyanins in the sample. For confirmation Amyl alcohol test was used. A mixture of equal quantities of dil. HCl, sodium bisulfite and sodium acetate prepared in a test tube. To this mixture was added 2ml of aqueous stock solution of *E. prostrata* and *E. granulata*. The samples with mixture were heated and after cooling 2ml of amyl alcohol added with gentle shaking. The appearance of red/violet/blue or scarlet coloration confirmed the presence of anthocynins.

Detection of coumarins

5 ml of stock solution of *E. prostrata* and *E. granulata* were heated in boiling water and a filter paper strip soaked by dipping in 1N NaOH was held for some time above the vapors from the samples. The filter paper was then checked for fluorescence in UV light. Yellow fluorescence shows that coumarins are present in the sample (Khan *et al.*, 2011).

Detection of steroids

Bahuguna (2007) technique was followed for detection of steroids. 0.5mgs of the extracts of *E. prostrata* and *E. granulata* were dissolved in 2ml of acetic anhydride in a test tube and then 2ml of H_2SO_4 was added. The appearance of violet coloration that changed to green or blue showed the presence of steroids. To confirm this, to 1ml of aqueous stock solution of *E. prostrata* and *E. granulata* extracts 10ml of chloroform added in a measuring cylinder and 11ml of H_2SO_4 added slowly from the sides of the cylinder. The formation of red coloration in chloroform layer and yellow coloration with green fluorescence in H_2SO_4 layer confirms the presence of steroids in the sample.

2. Quantitative analysis

Analysis for total phenolics, flavonoids, Alkaloids, tannins, glycosides and saponins were done for roots, stem, leaves and seed samples following the standard methods of different authors as follows.

a. Quantification of Total Phenolics

Spectrophotometric method used by Falodun & Irabor (2008) () was followed for this purpose. For making a calibration curve different dilutions of Gallic acids ie 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1mg/ml were prepared using serial dilution method. In a similar way 1mg/ml of *E. prostrata* and *E. granulata* were prepared from stock solution by serial dilution method were further diluted serially into 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1mg/ml. From each of the above sample 0.5ml was treated with 2.5ml of Folin-Ciocalteu's reagent (tenfold diluted). To each sample 2.0ml 0f 7.5% sodium carbonate was added. The reagents were allowed to react for 30 minutes. The absorbance for all samples was noted at 760nm. The concentration of phenolics was expressed as gallic acid equivalent (GAE). The analyses were performed in triplicate.

b. Quantification of Flavonoids

The technique of Chang *et al* (2002) was followed for flovonoids. For making a calibration curve different dilutions of quercetin i.e. 100, 90, 80, 70, 60, 50, 40, 30, 20, 10µg/ml were prepared in 80%ethanol using serial dilution method. Similarly 1mg/ml of *E. prostrata* and *E. granulata* were prepared from stock solution by serial dilution method and were further diluted serially into 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2,

0.1mg/ml. To 1ml of each sample above 200µl of 1mM potassium acetate, 200µl of 10% $AlCl_3$ solution and 5.6ml dist. H_2O added and let to react at room temperature for 30mnts. The absorbance was measured at 415nm and the absorbance value of sample dilution that comes in the range of quercetin calibration curve was plotted in the graph for finding the value of flavonoids in the sample. The experiment was carried in triplicate for each sample and water was used instead of $ALCl_3$ for blank reading. The result was expressed as quercetin equivalent.

c. Quantification of Alkaloids

Spectrophotometric method used by Sreevidya & Mehrotra (2003) was followed. For making a calibration curve different dilutions of Papaverine, i.e. 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100µg/ml were prepared in 95% ethanol (in 2% acetic acid) using serial dilution method. Similarly 1mg/ml of *E. prostrata* and *E. granulata* were prepared from aqueous stock solution by serial dilution method and were further diluted serially into 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1mg/ml. 5ml of each of the extract samples were acidified with HCl and 2ml Dragendorff's reagent added to it. The precipitate formed was separated by centrifugation and washed with 70%ethanol. The ppt was then mixed with disodium sulfide solution and a brownish black ppt formed was then separated by centrifugation and dissolved in 2ml HNO_3 by slight warming. Dist. H_2O was added to raise the final volume to 10ml. from this solution 1 ml was mixed with 5ml of thiourea and absorbance was measured at 436nm. The absorbance value of sample was plotted in standard Papaverine calibration curve for determining the value of alkaloids. The experiment was carried in triplicate for each sample and nitric acid and thiourea solution was used without plant sample for blank reading. The result was expressed as papaverine equivalent.

d. Quantification of Tannin

Using spectrophotometric method, the procedure of Okwu & Josiah (2006) with minor modification was adapted. The calibration curve for different dilutions of tannin acid i.e. 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100µg/ml was prepared in dist. H_2O using serial dilution method. Likewise 1mg/ml of *E. prostrata* and *E. granulata* were prepared from aqueous stock solution by serial dilution method and were further

diluted serially into 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1mg / ml. 0.1M $FeCl_3$ was prepared in 0.2N HCl and 0.008M potassium ferrocyanide. To 5ml of each of the extract samples were added 3ml of 0.1M $FeCl_3$ prepared and let to react for 10 minutes. The absorbance was measured at 120nm. The absorbance values of samples were plotted in tannic acid calibration curve. The quantity of alkaloids in the sample was determined from this graph. The experiment was carried in triplicate for each sample and dist. H_2O was used without plant sample for blank reading. The result was expressed as tannic acid equivalent (TAE).

e. Quantification of Glycosides

Spectrophotometric method of Qasheesh (2012) with minor modification was followed. For making a calibration curve different dilutions of digitoxin i.e. 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100µg/ml were prepared in 10%ethanol using serial dilution method. similarly 1mg/ml of *E. prostrata* and *E. granulata* were prepared from aqueous stock solution by serial dilution method and were further diluted serially into 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1mg/ml. 10ml of each of the extract samples were added to 10ml 0f Baljet's reagent, diluted with 20ml of dist. H2O and the reaction mixture was allowed at room temperature for 60 minutes developing color. The intensity of the orange-red color was measured by reading absorbance at 495nm. The absorbance value of sample was plotted against standard digitoxin calibration curve and the value of glycosides was determined for the sample. The experiment was carried in triplicate for each sample and ethanol alone without plant sample was used for blank reading. The result was expressed as digitoxin equivalent.

f. Quantification of Saponins

Saponins were quantified through spectrophotometric, using Vanillin–Sulfuric acid technique of Shiau *et al* (2009) with minor modification. For making a calibration curve different dilutions of quillaja saponin i.e. 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100µg/ml were prepared in 10% dist. H_2O using serial dilution method. Different dilutions of 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1mg / ml from stock solution of *E. prostrata* and *E. granulata* were also prepared in dist. H_2O. To 1ml of each of the extract samples were added to 1ml 0f vanillin 8% solution and then 10ml of 72% H2SO4 in a

test tube. The reaction mixture was thoroughly mixed on ice, then warmed to 60C0 for 10mints and lastly cooled in ice cold water. The intensity of the color developed was measured by reading absorbance 535nm. The absorbance value of sample dilution that comes in the range of quillaja saponin calibration curve was plotted in the graph for finding the value of saponin in the sample. The experiment was carried in triplicate for each sample and dist. H2O alone without plant sample was used for blank reading.

4. Mineral composition

For mineral analysis atomic absorption was used following the method of Aslam *et al* (2005) with minor modification. From stock solution of all elements working standards were prepared in 2N HNO_3. The elements analyzed were Fe, Mn, Zn, Cu, Mo, Ca, P, Mg, S, N, K, Na, Cl^-, Se, and sulfates. Using different dilution of working solution a calibration curve was prepared by noting absorbance versus concentration. 2gms powdered plant and plant parts (roots, stem, leaves and seeds) of the two plant species *E. prostrata* and *E. granulata* were subjected to ashing by exposing to high temperature in an electric oven at 550^0C in a clean porcelain crucible. The ash so produced was dissolved in 10ml of 2N HNO_3 and let to dissolve for 12hrs. After an overnight incubation the sample was heated until the production of reddish brown fumes comes to an end. Then 4ml of 67% HClO4 was added and heated for 5mnts. At the end 10ml aquaregia was added and heated to evaporate. The total volume of the sample was raised to 250ml with de-ionized water. The sample was subjected to Atomic Absorption Spectroscopy and the reading noted. The absorbance for each element was put in to the calibration curve and the concentration determined. Same procedure was used for analyzing soil sample.

5. Heavy metals analysis

Heavy metals, including Hg, As, Pb, Cd, Co, Cr, Ag, Mo, Ni, Sn were analyzed in plants (collected from three habitats), plant parts and soil sample using atomic absorption spectroscopy. The Powder plant material of *E. prostrata* and *E. granulata* were used for analysis following Khan *et al* (2008) with minor modification. From stock solution of the elements working standards for all elements were prepared in 2N HNO_3. To make a

calibration curve different dilution of working solution was prepared. A calibration curve was prepared by noting absorbance versus concentration. Using electric oven 2gms powdered plant and plant parts (roots, stem, leaves and seeds) of *E. prostrata* and *E. granulata* were subjected to ashing at 550^0C in a porcelain crucible. The ash so produced was dissolved in 10ml of 2N HNO_3 and let to dissolve for 12hrs. After an overnight incubation the samples were heated until the production of reddish brown fumes comes to an end. Then 4ml of 67% $HClO_4$ was added and heated for 5minutes. At the end 10 ml aquaregia was added and heated to evaporate. The total volume of the sample was raised to 250ml with de-ionized water. The sample was subjected to Atomic absorption spectroscopy (Perkin–Elmer 1100 B, USA) and the readings were noted. The absorbance for each element was put in to the calibration curve to determine the concentration. For soil Chehregani and Malayeri (2007) were followed. Soil from all the three habitats were collected from rhizosphere around the plant in three separate test tubes. The samples were the oven dried (at 70 0C for 72 hours), pulverized (using mortar and pestle) and sieved though 2mm sieve. one gram from each were used for heavy metals. each soil sample was digested with 20ml of 65% boiling nitric acids until the fume production ceased. They were then cooled and make the volume 100ml with deionized water. 20ml from these samples were then used for heavy metal analysis using Atomic Absorption.

6. Antidiabetic Activity

The diabetes induce rabbits method of Selvan *et al* (2008) with minor adjustments was followed. Healthy local strain of *Oryctolagus cuniculus* (Rabbits) were purchase from the local market. These animals were allowed for 10days in the experimental laboratory having free access (*ad libitum*) for food and water. The rabbits were given normal diet (grass and clean drinking tap water) during experimental period. Thereafter rabbits having body weight of 1200 to 1500gms, both male and non-pregnant female were selected for this activity. Glucose level was measured spectrophotometrically using kit method of Analyticon (Germany). 0.5ml of blood was collected each time from central ear artery and serum separated for analyzing blood glucose levels (BGL). Thirty rabbits were selected and marked for diabetization. Before administration of Alloxan monohydrayes Blood glucose level was measured for each rabbit. The rabbits were then

administerd 120mg/kg bw alloxan monohydrates in marginal ear vein in the morning after overnight fasting. After 48hrs the blood glucose level of the surviving rabbits was determined. The rabbits having BGL more than 180mg/ml were selected for further experiment. The experiment was repeated for acquiring the desired number of diabetic animals for experiment.

Experimental strategy: seven groups of five rabbits in each was prepared having the following purposes

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 10 mg / kg glibenclemide)

Group 3 = Normal tested (received 200 mg / kg EG extract)

Group 4 = Normal tested (received 200 mg / kg EP extract)

Group 5 = Streptozotocin treated negative Control (received empty capsule shell)

Group 6 = Streptozotocin treated positive Control (received 10 mg / kg glibenclemide)

Group 7 = Streptozotocin treated tested (received 200 mg / kg EG extract)

Group 8 = Streptozotocin treated tested (received 200 mg / kg EP extract)

Before administering the drug and the extract to the subject, blood samples were collected and analyzed for blood glucose level. Plant extract and Glybenclamide were weighed and enclosed in gellatin capsules for each rabbit. The capsules with sample were administered orally followed by sufficient water with the help of wash bottle. After the treatment blood samples were collected at 0 hour, 1/2hr, 1hr, 2hrs, 4hrs, 8hrs and 24hrs 48 and 72 hours to measure the blood glucose level. **Statistical analysis:** The results were tabulated for statistical evaluation using graphpad prism.

7. Antilipidemic activity

Myocardial infarction induced rabbit's method was used following Castro *et al* (2009) with slight changes. Rabbits were prepared and treated in the same way as for the above experiment. Total cholesterol, high density lipids HDL, low density lipids LDL and triglycerides TG levels were measured spectrophotometrically using kit method of Analyticon (Germany).

Inducing myocardial injury (MI): blood samples were taken and analyzed for lipid profile from those 15 rabbits selected to induce myocardial injury. Isoprenaline were prepared in normal saline and 20mg/kg were administered intra-peritoneally to all 15 rabbits for 2days to induce myocardial injury. After 48 hours blood samples were again collected and tested for lipid profile. The rabbits with increased serum lipid profile were selected for further experiment.

Experimental strategy: fourteen groups of five rabbits in each was prepared having the following purposes

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 3mg/kg resveratrol)

Group 3 = Normal tested (received 200 mg / kg EG plant extract)

Group 4 = Normal tested (received 200 mg / kg EP plant extract)

Group 5 = Isoprenaline treated negative Control (received empty capsule shell)

Group 6 = Isoprenaline treated positive Control (received resveratrol)

Group 7 = Isoprenaline treated tested (received 200 mg / kg EG plant extract)

Group 8 = Isoprenaline treated tested (received 200 mg / kg EP plant extract)

Group 9 = Diabetic negative control (received empty capsule shell)

Group 10 = Diabetic positive control (received resveratrol)

Group 11 = Diabetic tested (received 200 mg / kg EG plant extract)

Group 12 = Diabetic tested (received 200 mg / kg EG plant extract)

Group 13 = Preventive control (received 200 mg / kg EG plant extract along with isoprenaline)

Group 14 = Preventive control (received 200 mg / kg EP plant extract along with isoprenaline)

0.5ml of blood was collected each time from central ear artery and serum separated for analyzing lipid profile. Drugs and extracts were given enclosed in gelatin capsules. The capsules with sample were administered orally with the help of sufficient amount of water using wash bottle. After administering the extract, 0.5ml blood samples were collected after 1hour to measure the lipid profile. The levels of lipids were also measured at 24, 48 and 72hours after the dose.

Statistical analysis: Graphpad prism was used for analyzing the results.

8. Hepatoprotective activity

The carbon tetrachloride induced liver damaged rabbit's method was used following El-Gazzar *et al* (2009) with minor adjustments. The rabbits were obtained and acclimatized to lab environments as in above experiment.

Inducing liver injury: Blood samples were taken and analyzed for liver health indicator from those 15 rabbits selected to induce liver injury. Rabbits were administered with single dose of 2ml of 50% carbon tetrachloride in paraffin oil (v/v) subcutaneously. After 24 hours blood samples were again collected and tested for liver health indicator. The rabbits with abnormal liver health indicator were selected for further experiment.

Experimental strategy: ten groups of five rabbits in each was prepared having the following purposes

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 100 mg/kg silymarin)

Group 3 = Normal tested (received 200 mg / kg EG plant extract)

Group 4 = Normal tested (received 200 mg / kg EP plant extract)

Group 5 = CCl_4 treated negative Control (received empty capsule shell)

Group 6 = CCl_4 treated positive Control (received 100 mg/kg silymarin)

Group 7 = CCl_4 treated tested (received 200 mg / kg EG plant extract)

Group 8 = CCl_4 treated tested (received 200 mg / kg EP plant extract)

Group 9 = Preventive control (received 200 mg / kg EG plant extract along with CCl_4)

Group 10 = Preventive control (received 200 mg / kg EP plant extract along with CCl_4)

1.0ml of blood was collected each time from central ear artery and serum separated for analyzing liver health indicators. Total protein, Albumin, billirubin, creatinin, SGPT and SGOT levels were measured spectrophotometrically using kit method of Analyticon (Germany). Drugs and extracts were administered orally with plenty of water. After administering the drug and extract blood samples were collected after 1hour to measure

the liver health indicators (billirubin, creatinine, SGPT, SGOT, albumin, and total protein analysis). Analyses were repeated at 48 and 72 hours after the dose.

Statistical analysis: The results were evaluated with MS excel and graphpad prism software for.

9. Effects on bone health

The corticosteroid induced osteoporotic rabbit's method of Mirvish (1992) with slight changes was followed. The rabbits were handled and prepared for experiment in the same way as in previous experiment.

Inducing osteoporosis: Blood samples were taken and analyzed for bone resorption indicators (ALP, calcium and phosphates level) from 15 rabbits selected bone resorption. To induce osteoporosis the rabbits received 3.12mg/kg dexamethasone daily during experimental period of 20 days. PTH 25µg/kg daily was administered to prevent osteoporosis. Blood samples were again collected and tested for ALP, calcium and phosphorus levels.

Experimental strategy: Eight groups of five rabbits in each were prepared having the following purposes.

Group 1 = Normal negative Control (received empty capsule shell daily)

Group 2 = Normal positive Control (received 15µg/kg PTH daily)

Group 3 = Normal tested (received 200mg/kg of EG extract daily)

Group 4 = Normal tested (received 200mg/kg of EP extract daily)

Group 5 = Osteoporosis induced –ive control (received 3.12mg/kg dexamethasone daily)

Group 6 = Osteoporosis induced +ive control (received 3.12mg/kg dexamethasone and 15µg/kg PTH daily)

Group 7 = Osteoporosis induced tested (received 3.12mg/kg dexamethasone and 200mg/kg of EG extract daily)

Group 8 = Osteoporosis induced tested (received 3.12mg/kg dexamethasone and 200mg/kg of EP extract daily)

1.0ml blood samples were collected at day 1, 5, 10, 15 and 20 to measure the level of bone health indicators (ALP, calcium and phosphates). These were measured spectrophotometrically using kit method of Analyticon (Germany).

Statistical analysis: The results were tabulated for statistical evaluation using MS excel and graphpad prism.

10. Effects on blood electrolytes

The method of Ae *et al*, (2009) with slight adjustments was used. Rabbits were obtained and prepared for the experiment in the same way as done for the previous experiment.

Experimental strategy: ten groups of five rabbits in each was prepared having the following purposes

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 0.5mg / kg furosemide)

Group 3 = Normal tested (received 50 mgs / kg EG plant extract for five days)

Group 4 = Normal tested (received 100 mgs / kg EG plant extract for five days)

Group 5 = Normal tested (received 150 mgs / kg EG plant extract for five days)

Group 6 = Normal tested (received 200 mgs / kg EG plant extract for five days)

Group 7 = Normal tested (received 50 mgs / kg EP plant extract for five days)

Group 8 = Normal tested (received 100 mgs / kg EP plant extract for five days)

Group 9 = Normal tested (received 150 mgs / kg EP plant extract for five days)

Group 10 = Normal tested (received 200 mgs / kg EP plant extract for five days)

0.5ml of blood was collected from each rabbit before and after administering the drugs for 5 days. The blood sample was collected from central ear artery and serum separated for analysis. Sodium and potassium were measured with flame photometer method at 590nm and 770nm respectively while chloride was measured by titration method of mercuric nitrate developed by Schales (Ofem *et al*, 2010). Furosemide and plant extracts were weighed and enclosed in gelatin capsule. The capsules with sample were administered orally followed by plenty of water with the help of wash bottle.

Statistical analysis: the data obtained was processed with MS excel and graphpad prism.

11. Effect on hematological parameters

1. Antianemic activity

Anemia induced rabbits were used for evaluating anti-anemic potential of *E. prostrata* and *E. granulata* following the method of Iwalewa *et al* (2009). Healthy rabbits purchased from local market were used for this experiment. These rabbits were provided optimal conditions to acclimatize in the lab. Anemia was induced in rabbits using phenylhydrazine hydrochloride (Berger, 2007).

Inducing anemia: Anemia was induced in rabbits by injecting phenylhydrazine hydrochloride (20mg/kg in aqueous solution) intraperitonially (Berger, 2007) which Reduces RBCs by 50% and Hb by 60%.

Experimental strategy: ten groups of five rabbits in each was prepared having the following purposes

Group 1 = Normal negative Control

Group 2 = Normal positive Control (received Iron and B_{12} preparation)

Group 3 = Normal tested (received 200 mg / kg EG extracts)

Group 4 = Normal tested (received 200 mg / kg EP extracts)

Group 5 = phenylhydrazine-HCl treated negative Control

Group 6 = phenylhydrazine-HCl treated positive Control

Group 7 = phenylhydrazine-HCl treated tested (received 200 mg / kg EG extracts)

Group 8 = phenylhydrazine-HCl treated tested (received 200 mg / kg EP extracts)

Group 9 = Preventive control (received 200 mg / kg EG extracts along with PHZ)

Group 10 = Preventive control (received 200 mg / kg EP extracts along with PHZ)

1ml of blood was collected each time from central ear artery and was EDTA treated to prevent clotting and studying blood morphology and count. Red blood cells **(**RBCs), White blood cells (WBCs), Platelets count (PLC) or thrombocytes were count microscopically, hemoglobin concentration (Hb) concentration was determined with Hb meter, Packed cell volume (PCV) was measured with glass capillary while Mean corpuscular hemoglobin MCH and Mean corpuscular hemoglobin concentration MCHC were calculated from the data of Hb and PCV.

Statistical analysis: the data obtained was processed with MS excel and graphpad prism.

2. Immuno-modulatory activity

The method of Iwalewa *et al* (2009) was adapted with some adjustments. Local strain of rabbits was used for this activity. Before experiment the rabbits were let to live in laboratory environments for acclimatization. The rabbits were treated with different doses of plant extracts for five days and the impact was noted on level of total leukocyte count (TLC), neutrophils, eosinophil, basophils, monocytes, lymphocytes and thrombocytes.

Experimental strategy: ten groups of five rabbits in each was prepared having the following purposes

Group 1 = Normal negative Control received empty capsule shell for five days

Group 2 = Normal positive Control (received 10 µg / kg filgrastim for five days)

Group 3 = received 50 mgs / kg EG extract for five days

Group 4 = received 100 mgs / kg EG extract for five days

Group 5 = received 150 mgs / kg EG extract for five days

Group 6 = received 200 mgs / kg EG extract for five days

Group 7 = received 50 mgs / kg EP extract for five days

Group 8 = received 100 mgs / kg EP extract for five days

Group 9 = received 150 mgs / kg EP extract for five days

Group 10 = received 200 mgs / kg EP extract for five days

For measuring immunological profile 1.0 ml blood was collected from rabbits before the administration of extract dose and after five days of treatment with extract.

Statistical analysis: The data were tabulated in MS excel and statistically analyzed using graphpad prism.

12. Antibacterial activity

Preparation of Nutrient agar (NA) Plate: 11.5gms/500ml of Nutrient agar plates was prepared using manufacturer instructions. The agar was sterilized in autoclave at 121C for 20mnt and was transferred to water bath to bring it the desired 45^0C temperature and then 25ml of it was poured into sterilized petridishes to solidify at room temperature. The solidified agar plates were stored for activity.

Nutrient broth (NB) preparation: 1gm / 125ml Nutrient broth was prepared and sterilized as above in 500ml glass bottle in autoclave. It was then dispensed to culture tubes aseptically as needed per procedure.

100mg/ml of plants of EP and EG extract was dissolved in 1ml Dimethyl sulfoxides (DMSO).

10 mg / ml streptomycin was prepared in distilled water.

Technique: Radial diffusion (well diffusion) technique developed by Kirbybauer was used following Hoskeri *et al* (2010). Reference bacterial strains of ATCC were obtained from PCSIR lab Peshawer and were grown in nutrient broth at 37^0C for 24hrs in culture tubes. *Klebsiella pneumonia* (700603ATCC), *Bacillus cerus* (QUI-ATCC), *Citrobacter frundii* (QUF-ATCC), *Streptococcus aureus* (25923ATCC), *Enterococcus faecalis* (29212ATCC), *Pseudomonas aeruginosa* (27853ATCC), *Escheritia coli* (25922ATCC) and *Serratia marcscens* were used as the test organisms. Turbidity of the culture was compared with McFarland turbidity equivalent. After solidifying, the bacterial culture was spread equally with cotton swab on agar. Wells were dug in the media using a sterile 0.6mm cork borer at suitable distance. The wells were filled with plant extracts, positive control (cefuroxime) and negative control (DMSO). The petridishes were incubated at 37^0C. After 24 hours the plates were checked for presence of inhibition zone. The sizes of the inhibition zones were measured. The procedure was performed in triplicate for each bacterial strain.

Minimum Inhibitory Concentration (MIC)

Broth dilution technique was used, following Owolabi *et al* (2009) with minor adjustments. From stock solution (100mg/ml) of *E. prostrata* and *E. granulata* ten different dilutions of 100, 90, 80, 70, 60, 50, 40, 30, 20, 10mg/ml were prepared in culture tubes. For Positive control 10mg/ml streptomycin and for negative control DMSO was used. The bacterial culture was diluted with normal saline (0.9% NaCl w/v) to bring the turbidity equal to McFarland standard ($1.5x10^8$ CFU/ml). From each extract sample 0.5ml was added to a series of 10 culture tubes in triplicate. 0.5ml streptomycin was added to positive control and DMSO to a negative control. To each of these culture tube 2ml of nutrient broth and a loop full of bacterial culture was added and incubated at $37C^0$.

After 24 hours the tubes were checked for bacterial growth by observing visible turbidity. The lowest concentration at which no bacterial growth occurred was recorded as MIC against that bacterial strain. This procedure was repeated for all eight strains of bacteria.

Minimum Bactericidal Concentration

The bioassay was conducted by using Doughari (2006) method with slight changes. From the above experiment carried for MIC, culture tubes with no visible turbidity were selected and were spread on fresh nutrient agar plates. The plates were incubated for 24hours at $37C^0$ and were checked for the growth of bacterial colonies. The concentration at which bacterial colonies had grown was considered as bactericidal concentration.

13. Antifungal Activity

Preparation of Media: 19.5gms/500ml of potato dextrose agar (PDA) plates was prepared using manufacturer instructions. The agar was sterilized in autoclave at 121C for 20mnt and was transferred to water bath at 45^0C. 25ml of this agar was poured into sterilized petridishes to solidify at room temperature. The solidified agar plates were stored for activity.

Similarly sabouraud dextrose broth (SDB) was preparation by dissolving 6gms in 200ml of water as instructed by the manufacturer. The broth was sterilized in 500ml glass bottle in autoclave and was dispensed to culture tubes aseptically as needed per procedure.

Technique: Kerby Bauer technique was used, following Jagessar *et al* (2007) to check antifungal activity *E. prostrata* and *E. granulata*. Four reference fungal strains of ATCC including *Aspergillus flavus* (QUF-ATCC), *Aspergillus niger* (QUI-ATCC), *Aspergillus parasiticus* (QUF-ATCC) and *Fusarium oxysporum* (1020ATCC) were obtained from PCSIR lab Peshawer and used in this activity. 100mg / ml of plants of *E. prostrata* and *E. granulata* extract were prepared in DMSO. After growing in culture tubes the turbidity of culture was compared with McFarland turbidity standard and was spread equally over the media. For testing samples, wells were dug at suitable distance in the media using 0.6mm cork borer. The wells were filled with already prepared different plant extracts, positive control (Fluconazole) and negative control (DMSO). The

petridishes were transferred to incubator set at 37^0C and let there for growth. After 24 hours incubation the plates were checked for presence of inhibition zone and the size of zone was measured. Sensitivity of each fungal strain was checked in triplicate.

Minimum Inhibitory Concentration (MIC)

Agar dilution method of Tra *et al* (2008) with slight changes was followed. For these activity ten different concentrations of 100, 90, 80, 70, 60, 50, 40, 30, 20, 10mg/ml of *E. granulata and E. prostrata* were prepared in PDB. The tubes were inoculated with fungal culture having McFarland turbidity and kept at $30C^0$ for 48hrs. Fungal growth was checked visually at the end of incubation period. The extract concentration at which no growth occurred in broth tube was considered as MIC for the fungal strain. The protocol was carried in triplicate.

Minimum Fungicidal Concentration

The method of Mann *et al* (2008) with some adjustments was followed. From the experiment of MIC, culture tubes with no fungal growth were further inoculated on fresh PDA plates. The plates were incubated for 48 hours at $37C^0$ and were checked for fungal growth. The concentration at which no fungal growth occurred was considered as fungicidal concentration.

Data analysis: the data so obtained was subjected to graphpad prism for ± SD.

14. Antioxidant Effects

Using Diphenyl picrylhydrazyl (DPPH) method of Ara & Nur (2009) antioxidant activity of whole plant, roots, stems, and leaves of *E. granulata and E. prostrata* were investigated. For making a calibration curve different dilutions of Ascorbic acid i.e. 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1mg/ml were prepared in dist. H_2O. Similarly 1mg/ml of *E. granulata and E. prostrata* were prepared and serially diluted into 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1mg/ml. A 0.004% w/v solution of DPPH was prepared and 2ml from this was mixed with 2ml of each sample as well as with ascorbic acid. The solution was well mixed by slow shaking and then kept for 30mnts at room temperature. At the end of incubation time the intensity of the color was measured at 517nm using spectrophotometer. A calibration curved was made for standard ascorbic acid. The

absorbance values of the samples were noted and compared with standard curve. Free radical scavenging properties of the test samples was interpreted by comparing the results with ascorbic acid as a positive control and solving the values using the formula

%cent quenching = $\left(\frac{Ac-As}{Ac}\right)$ 100

Where Ac is Absorbance by blank control, As absorbance by sample.

15. Antitumor activity

Agrorbacterium tumefacien induced tumor technique, used by McLaughlin (1998) and Islam *et al* (2009) with some adjustments was followed.

Collection of *Agrorbacterium tumefacien*: Crown galls were collected from different infected plants inside Peshawar University Campus. Isolated crown gall was sterilized with detergents, sterilized water and was crushed using aseptic techniques. The crushed crown gall was inoculated into LB broth for 24hrs and was streaked on LB (Luria Bertani) agar plate for isolation. *Agrobacterium tumefacien* was identified from the shape, size, color and form of its colony. The bacterium was re-inoculated from ten identical colonies into a culture tube and was confirmed after biochemical tests. a loopful from the bacterial colony grown in LB agar plat was transferred aseptically into LB broth and let it grow for 24hrs at $30C^0$. Growth of the bacterial culture was confirmed from the turbidity and color of the broth.

Preparation of Potato and carrots Discs: healthy potato tubers were first sterilized using 0.1 % $HgCl_2$ solution and then a tissue cylinder was cut with the help of a sterilized cork borer. Using aseptic technique the initial 1cm end was cut discarded and rest of the cylinder was sliced into many uniform size cylinders. The same procedure was repeated for preparing carrots discs also.

Preparation of LB agar Plate: 1.5gms/100ml of Luria Bertani agar (LB) plates were prepared using manufacturer instructions. The agar was sterilized in autoclave and 25ml of it was poured into each petridish at 45C0. The plate were allowed to solidify and stored for activity.

Technique: 100mg/ml of extracts of both *E. granulata and E. prostrata* were prepared in DMSO. Five sterilized potato discs were placed aseptically at equal distance from each other on LB agar plates for every test sample. From stock solution of extract 50, 25, 12.5, 6.25mg/ml was prepared so that on loading 10µl of this solution on a potato disc the discs received 1mg, 500µg, 250µg, 125µg and 62.5µg/ml of the extract. The discs were inoculated with 10µl of *Agrobacterium tumefaciens* culture having turbidity equal to McFarland standard (10^8 CFU / ml). Vincristine (a known anticancer drug) was used as positive and DMSO as negative control. Petridishes were sealed with parafilm to prevent water lost and incubated at room temperature for three weeks. The experiment was performed in triplicate for each test sample and the same procedure was repeated for carrot discs method. At 21^{st} day of incubation the discs were checked visually for tumor growth. Those having no visible growth were checked under magnifying glass and treating with Lugol's solution. As the tumor cells lack starch these cells can't be stain with logul's solution and hence can be easily seen with magnifying glass or dissecting microscope.

Data analysis: As compared to standard anti cancer drug, the sample having 20% or more inhibition may be considered as significant inhibition. The formula used for %cent inhibition is

% Inhibition = 100 – (No of tumors on sample/ No of tumors on control) 100

16. Cytotoxic Activity

Brine shrimp lethality Assay (BSA) used by Mclaughlin (1998) was followed with some adjustments. Artificial sea water (ASW) was prepared by 3.8gms of sea salt in 100ml of distilled water.

Eggs Hatching: A 20 x 8 cm light proof tray was divided with perforated plastic band into two 1:3 unequal portions. The tray was filled with enough ASW to cover the hole in the partition wall. The entry of light to larger part was blocked with a plastic cover. 10mg of Brine shrimp (*Artemia salina*) eggs were added to the larger portion of the tank and covered with a lid to prevent light. The open end of

the tank was illuminated with lamp so that phototrophic nauplii (Brine shrimp larvae) may move towards the light through the pore in the partition. The setup was left to hatch at room temperature for 48hrs.

Technique: 100ml ASW was prepared in a beaker, filtered and poured in to the water tank. From stock solution (100mg/ml) of *E. granulata and E. prostrata*, three dilutions of 5000 µg, 500µg and 50µg/ml in DMSO were prepared. 1ml from each of the above samples was poured into test tubes and allowed to evaporate to dryness. After dryness 3ml of ASW was added into each test tube and 10 nauplii were added with the help of disposable Pasteur pipette. The final volume in a test tube was raised to 5ml with ASW so that the tube contain 1000, 100 and 10µg/ml of test samples. 0.30mg/ml potassium dichromate was used as positive control while DMSO as negative control. The experiment was performed in five replicate and was left at room temperature for 24hrs. After 24hrs the nauplii in positive control, negative control and test sample were counted in all test tubes and percent toxicity calculated.

17. Phytotoxic Activity

Phytotoxicity was evaluated on Lemna growth, radish seeds germination and radish roots length determination.

Lemna bioassay

Method used by Atta-ur-Rahman (2005) was followed for this bioassay. *Lemna minor* having three and two fronds were selected from local pond for this experiment. The pond water from which lemna was collected was filtered sterilized in autoclave and used as growth media for experimental plant. From stock solution (100mg/ml) of *E. granulata and E. prostrata*, three dilutions of 5000 µg, 500 µg and 50 µg/ml in DMSO were prepared. 4ml from each of the above samples was poured into 50ml beaker and allowed to evaporate to dryness. After dryness, 15 ml of growth media was added into each beaker. The pH was adjusted to 5.5 and 10 *lemna minor* each having three fronds was added with the help of a forceps. The final volume of media in a beaker was raised to 20 ml with growth media so that the

media contain 1000, 100 and 10 µg/ml of test samples. Glyphosate (a well known broad spectrum herbicide) was used as positive control while growth media without any sample as negative control. All the beakers were covered with aluminum foil to minimize evaporation. The experiment was performed in five replicate and was left at room temperature. The plants were observed daily for 1week. After 1week the lemna fronds in positive control, negative control and test sample were counted in all beakers and recorded for percent toxicity calculations.

$$\text{Mortality\%} = \left[\frac{100 - Number\ of\ fronds\ in\ test\ samples}{Number\ of\ fronds\ in\ negative\ control}\right] \times 100$$

Radish seeds phytotoxicity

McLaughlin (1998) technique with some adjustments was adapted to check phytotoxicity of *E. granulata and E. prostrata*. To investigate toxicity against radish seeds, effect on seed germination and roots length were evaluated.

Radish seed germination: From stock solutions of *E. granulata and E. prostrata* two dilutions of 7.5mg/ml and 1mg/ml were prepared in DMSO. Filter paper discs of 9 cm were soaked with 5ml of each extract sample and were dried in an electric oven at $50C^0$. They were placed in 9cm petridishes. The discs were soaked again with 5ml of sterilized (autoclaved) tape water. Fifty radish seeds (surface sterilized with 0.1%HgCl3) were distributed at equal distance from each other in each plate. To avoid contamination the plates were sealed using parafilm and were let to grow at room temperature. The seeds were checked and noted daily for five days for growth. For negative control filter papers soaked and dried in DMSO was used. The experiment was performed in triplicate and at the end of incubation period the percentage inhibition of seeds growth calculated.

Roots length measurement: For determining the impact of extract on roots length the above experimental set up was repeated with extract concentration of 10 mg / ml and 1 mg/ml in DMSO. Unlike the above experiment here the increase in roots size was monitored on daily basis. Fifteen seeds were used in each experiment and the experiment was performed in triplicate. At the end of incubation period the average

growth of roots in extract samples and that of negative control was compared and %cent inhibition calculated.

18. Insecticidal activity

Insecticidal activity was determined using residual film toxicity bioassay, fumigant toxicity bioassay and repellency bioassay.

Residual Film Toxicity

The technique used by Alam *et al* (2009) was followed. For this experiment, *Tribolium castaneum* reared in the infested seeds, obtained from local market were used. 100mg / ml of extracts of both *E. granulata and E. prostrata* were prepared in DMSO. From stock solution (100mg/ml) of EP and EG ten different concentrations of 100, 90, 80, 70, 60, 50, 40, 30, 20, 10mg/ml were prepared in ten sterile test tubes in DMSO. From each sample 1 ml was evenly spread in each of ten petridishes. For positive control 1 ml lindane and for negative control 1ml DMSO was used. The petridished were let to evaporate so that a thin layer of the samples were left at the bottom. The quantity of the extract left per cm2 was determined by calculating area of petridish (18.26cm) using the formula πr^2 and then dividing the concentration over the area. Ten insects (*Tribolium castaneum*) were released into each plate and were incubated at room temperature. The protocol was carried in triplicate for all samples. The number of death insects was checked after 24hrs interval.

Toxicity of the sample was calculated using the formula

%cent toxicity = 100 – (number in (+) control-number in sample/number in (+) control) x100

Fumigant Toxicity

Waliwitiya *et al* (2005) technique with slight adjustments was followed for fumigant toxicity. Two concentrations (100 and 50 mg / ml) of *E. granulata and E. prostrata* were prepared in DMSO. Filter paper pieces of 2cm diameter were prepared with the help of a scissor and placed at the bottom 2cm vial. Each filter paper piece was loaded with 200µl of the samples and was let open to dry for 5minutes. For positive control 200µl of lindane and for negative control 200µl of DMSO was used. Ten insects

(*Tribolium castaneum*) were released into each vial and were screw tightened to let the vial and insects for 24hrs. The protocol was carried in triplicate for all samples. Mortality was determined by calculating the percentage of death insects.

Percent toxicity = number in control-number in sample / number in control x 100

Repellency Bioassay

The method used by Viglianco *et al* (2008) was followed. Ten different concentrations of *E. granulata and E. prostrata* were prepared in DMSO as in the above experiment. Filter paper pieces of half circle shape and 3cm radius were prepared with the help of a scissor so that the piece may cover half bottom of a 6cm petridish. The pieces were loaded with 200µl for each sample and let open to dry in air for 5mnts. Positive control was loaded with 200µl Lindane while negative control was loaded with 200µl DMSO. A similar piece having the same size and shape without the sample applied was then attached with adhesive to this half so that a complete circular disc of 6cm diameter was prepared half with and half without sample. Before placing the discs the petridishes were smear evenly with flour (diet for insect) and after placing the discs in the plates 15 insects were released into each. The movement of insects between the two half (sample treated and untreated) of the filter paper was observed and noted for 10hrs at 1hr interval. The protocol was carried in triplicate for both samples. Percent repellency was calculated from the number insects settled negative control and that on the sample applied.

Percent repellency = 2(%NCH-50)

Where NCH is percentage of insects on negative control half of filter paper disc. The positive results indicate repellency and the negative results show no repellency.

19. Reagents preparation

Dragendorff Reagent Preparation: To prepare the reagent solution A and solution B is prepared as: Solution A: 4:1of water and acetic acid solution is prepared and 1.7 g basic bismuth nitrate is added.

Solution B: Mix 40gms KI in 100ml of water.

Now 10ml each from A and B solution, 40ml acetic acid and 140ml of dist. H2O is mixed.

Molish reagent: 10% alcoholic solution of α-naphthol.

Benedict's reagents: for preparing 100ml of solution10 g of anhydrous sodium carbonate, 17.3 g of sodium citrate and 1.73 g of copper(II) sulfate pentahydrate in H2O.

Fehling's A: 6.9 g of CuSO4 in 100ml dist. H2O containing few drops of conc. H2SO4.

Fehling reagents B: 35 g of NaOH and 8.65g of sodium potassium tartarate in 250 ml H2O.

Barfoed's reagent: add 6.5 g of cupric acetate to 100ml of acetic acid in water.

Selwinoff'r reagent: in 100 ml of dilute 4N HCl dissolve 0.05 g of resorcinol.

Tollen's reagent: dropwise addition of sodium hydroxide to $AgNO_3$ solution in a test tube till precipitation is complete. After filtration dissolves the brown residue in NH_3 until a clear solution is obtained.

Bromine water: agitating 2-3 mL of bromine with 100ml of water in bottle.

Millon's reagent: Dissolve 10 grams of Hg in 20ml of hot conc. HNO3, and dilute the resulting solution with 30ml of H2O.

Ninhydrine: 0.2 g of ninhydrin in 100 ml of rectified spirit.

Mayer's reagent: dissolving 1.36gms of mercuric chloride and 5gms of potassium iodide in 100ml water.

Wagner's reagent: Dissolve iodine (2gms) and KI (6gms) in 100ml water.

Hager's reagent: 1g of picric acid in 100ml of dist. H2O and dissolves properly.

Folin-Ciocalteu's reagent: add mixture of sodium tungstate (10gms) and sodium molybdate (2.5gms) in 70 ml of water. Now add 5 ml of phosphoric acid (85%) and 10 ml of conc. HCl. Reflux for 10 hours. Add lithium sulfate (15gms), water (5ml), and bromine (1drop). Reflux for 15 minutes. Cool and bring volume to 100 ml with dist. water.

DPPH: 0.004% solution was prepared by dissolving 4mg of powder DPPH in 100ml ethanol.

Preparation of Logul's iodine: 5 % I2, 10 % KI in distilled water)

OBJECTIVES OF THE STUDY

Euphorbia prostrata and *Euphorbia granulata* are used for different ailments, locally in Swat and other areas of Pakistan. The present study was carries out with the following objectives.

- To uncover the nutritional status of the two plants and relate presence of different nutrients to their role in treating diseases.
- To analyze the plants for the presence of known phytochemicals and link their role to the physiological impacts produces in the consumer body.
- To find out the quantity of toxic heavy metals in the two plants and evaluate the possible toxicity to its consumers.
- To uncover the plant role in extracting toxic heavy metals from soil (Phytoextraction).
- To find out the role of plant in treating metabolic disorders like diabtes mellitus (DM), obesity (antilipidemic role), osteoporosis, acid base balance, optimizing and protecting the functions of liver.
- To test the plant for hematological (anemia) and immunological abnormalities.
- To investigate whether the plant have the possible role in inhibiting the growth of pathogenic microbes.
- To screen the plant for cytotoxicity, phytotoxicity and antitumor potential so that the possibility of anticancer drugs in the two plants is may be considered.
- Trying the two plants extracts against insects was also the objective of present research work.

CHAPTER 4

RESULTS AND DISCUSSIONS

1. Proximate analysis

Euphorbia granulata Forssk

Sufficient moisture is important for normal metabolic activities in the living cells. The adequate intake (AI) of water for a 70 kg person on average is 3.7 liters daily (NAS, 2005). Comparing the whole plant and plant parts, high moisture contents were recorded for seeds (Fig. 4). The high moisture contents in the seed might be an adaptation to the dry conditions through which the seed passes from its shedding to the next suitable condition for seed germination. The moisture contents ranged between 4.7% ± 0.25 (for roots) to 12.2% ± (for seeds) are shown in Fig. 4. Similar trend of high moisture was also reported by Shad *et al*. (2002) for *Fagonia arabica* (58.51%) that strengthen the present findings. The next high moisture contents were found in leaves (9.2 ± 0.26). Leaves need sufficient water for photosynthetic process and metabolic activities of young meristematic cells. Stem and roots is main medium for water flow and not to retain water.

The ash contents of a plant represent the inorganic mineral composition (Aberoumand, 2010; Folorunso &Modupe, 2007; Yusuf *et al*., 2007). Stem had more Ash contents than leaves, roots or whole plant. Plants need sufficient minerals for growth and metabolic activities in the growing parts. Stem is the main route for mineral carriage to the leaves. The maximum ash contents (12.1 ±0.36%) in the stem (Fig. 5) of *Euphorbia prostrata* indicated that major portion of stem was composed of organic combustible materials. While studying *Withania somnifera* Krishnamurthy & Sarala (2010) also found high ash contents in the stem. The next high ash contents (11.4 ± 0.208%) were recorded in the leaves which need it for a number of enzymatic activities to meet the need for high metabolic rate in the leaves. As seed contain a high percentage of energy rich lipids, carbohydrates and protein the inorganic mineral might be in low amount. This could be the reason for the least (6.1 ± 0.208%) amount of ash in the seeds.

The results for lipids analysis showed high amount (19.9 ± 0.351%) in the seeds, followed by stems and roots (3.7 ± 0.3%). Seeds had the high lipids contents (19.9 ±

0.35%). There was little variation between the whole plant and plant parts i.e. it was 3.7 ± 0.3 to 4.2 ± 2% in different samples (Fig. 6). Lipid contents in the seeds were 5 times higher than in the roots (3.7 ± 0.3%). The high lipids in seed are agreeable with lipids found in soya beans (Yusuf *et al*., 2007). This might be energy reservoir for the high energy needs of young embryo during germination. There is no recommended adequate intake (AI) value for total lipids; however 17 grams linoleic acid and 1.6 gram linolenic acidc is recommended AI (NAS, 2005). The lipid profile of seeds could be a possible source of essential fatty acids (EFA) that could cure and protect the health (Erdman *et al*., 2012; Gropper *et al*., 2009). Lipids also serve as energy reservoir, work as insulator for certain tissues and organs, and together with protein form the cellular components (Folorunso &Modupe, 2007). The EFA help in preventing many diseases (Erdman *et al*., 2012; Gropper *et al*., 2009). The optimum need for good health is that 30-50% of the total calories must be from lipids (Rinzler, 2009). Although, daily therapeutic dose of *Euphorbia granulata* is small yet it might be a source of EFA.

Fibers are important dietary constituents due to their role in water retention and in producing peristalsis in alimentary tract. It helps protect against colon and breast cancer, cardiac disease, diabetes and is source of energy for herbivores like ruminant (Folorunso &Modupe, 2007; Yusuf *et al*., 2007). National Academies of Sciences, or NAS, (2005) recommends 38 grams/day for adult male and 25 grams for female. During pregnancy and lactation these requirements raises to 28 and 29 grams, respectively. In the present study the fibers contents of the whole plant were 18.3 ± 0.68 % (Fig. 7). The maximum (27.8 ± 0.61%) and minimum (4.8 ± 0.289%) were noted for roots and seeds respectively (Fig. 4). High quantity of fibers in the roots was also pointed by Shad *et al*. (2002) in *Fagonia arabica*. The high fiber contents are important for roots to giving strong support and to absorb maximum water for plant. The optimum intake is 1-5 mg per serving (Rinzler, 2009). The quantity of fibers noted in *Euphorbia granulata* is sufficient to contribute towards the patient health.

NAS (2005) recommends 56 and 46 grams of proteins daily for adult male and female respectively. For female, additional 25 grams daily is recommended during pregnancy and lactation. Proteins are important body constituent that makes structural

components of cytoskeleton. Other functions of protein include biological catalysis (enzymes), immunity (immunoglobulin) and storage (ferritin). They are structural components (actin & myosin), cell receptors, and source of energy (Erdman *et al*., 2012; Gropper *et al*., 2009). It can be said that life is due to proper function of protein. High amount of proteins was recorded in seeds and the least in the stem (Fig. 8). The range was from 22.4 ± 0.379% (seeds) to 15.8 ± 0.306% (stem). Similar distribution pattern of high protein in seeds and leaves was also reported for *Fagonia arabica* by Shad *et al*. (2002) and for *Tamarindus indica* by Yusuf *et al*. (2007). The high protein contents in leaves might represent the high metabolic process in leaves, while in seed it is food source for future embryo. For good health 5-20% of the total calories must be from protein (Rinzler, 2009). Consumption of *Euphorbia granulata* for therapeutic purpose (4-5 grams/day) can't provide sufficient quantity of proteins. However, the plant could be a source of essential amino acids that will help in curing and saving the patient health (Erdman *et al*., 2012; Gropper *et al*., 2009).

Carbohydrates are quick source of energy for daily activities. Carbohydrates or nitrogen free extract (NFE) is an important dietary constituent. Beside source of energy, the body also uses it for the synthesis of other nutrients like fatty acids and amino acids. Cardiac glycosides and antibiotics are drugs. Mucopolysaccharides are ground substances for messenchymal cells (Erdman *et al*., 2012; Gropper *et al*., 2009). NAS (2005) suggested 130 grams carbohydrates daily both for adult male and female. During pregnancy and lactation 175 grams daily is recommended. It is further suggested that for good health 20-60% of the total calories must be from carbohydrates (Rinzler, 2009). The present results showed that the plant contained 39.9 ± 1.32% carbohydrates but the plant ingestion by the patient is not enough to provide healthy amount. The possible advantage could be that certain carbohydrates derivative in *Euphorbia granulata* might work as drug (Erdman *et al*., 2012; Gropper *et al*., 2009).The NFE was distributed in *Euphorbia granulata* in order as; stem > whole plant > leaves > roots > seeds (Fig. 9). The high value was noted in stem (41.4 ± 0.379%), while the low amount in seeds (34.6 ± 0.153%).

Acidity shows the various stable acids produced during metabolic cycle of a plant. Acidity plays role in synthesis, translocation and distribution of active metabolites and minerals (Krishnamurthy & Sarala, 2010). High acidity (pH 4.4) was noted for leaves (which might be due to synthesis of acidic metabolite during Kreb's cycle) and the least for stem (where acidity might b neutralized due to continuous flow of water to different parts) (Fig. 10). Total soluble solid (TSS) present in a plant represents the various biochemical in soluble form. It is an important indicator for assessing the maturity, and nutritional quality of a plant (Shahnawaz *et al*., 2009). The highest TSS value (0.5 ± 0.05) was noted for roots (the most mature plant part) while the seeds had the least TSS (0.1) amount (Fig. 11).

Energy requirements vary from person to person depends on the age, sex, body weight and type of activities. On average Estimated Energy Requirements (EER) for average person of 70 kg weight and 177 cm height is 3067 kcal/day. This need is comparatively less in female with 163 cm height and 57 kg weight which is 2403 kcal daily. The need further increases during pregnancy (NAS, 2005). Different food supply different calories (Gropper *et al*., 2009). Energy value of a plant may not necessarily reflect the actual energy obtainable because certain nutrients are not digestible and without contributing to the energy needs. The present study showed that whole plant and plant parts had high contribution to the calorific value in the carbohydrates form, followed by protein and the least from lipids (Fig. 12). This reflects the overall poor lipids in the plant. Earlier the during detection tests it was noted that all the different types of carbohydrate were present in the plant (Table 2) which could be due to the high carbohydrate composition of the plant. The overall nutritive value of the plant parts was noted for seed which contain high energy rich lipid in high amount. Although, not edible but *Euphorbia granulata* contained appealing quantities of proteins and carbohydrates.

For an adult man of 70 kg body weight and surface area 1.7 square meters, the basal requirements are 1632 C/24 hours (Erdman *et al*., 2012; Gropper *et al*., 2009). According to Rinzler (2009) an optimum source of food must provide 30-50% calories from fat, 5-20% protein and 20-60% carbohydrates (Rinzler, 2009), based on these figures, 489 - 816 kcal should be from fats, 8-326 Cal from and 326-979 Cal should be

obtained from carbohydrates. For required calories one has to eat more than 1 kg of this plant daily while therapeutic dose is 4-5 grams. As the plant is not an edible one and have an unacceptable taste, hence at therapeutic dose the plant is not a good source of energy.

***Euphorbia prostrata* Aiton**

Proximate analysis results of *Euphorbia prostrata*, including percent moisture, ash, lipids, fibers, proteins, nitrogen free extract (NFE), acidity, soluble solids and energy values are shown in Figs. 13 to 21.

The moisture contents of the two plants when compared, it was noted that *E. granulata* had 6.5% ± 0.36 moistures (Fig. 4) while *E. prostrata* was noted with 6.2% ± 0.2 (Fig. 13). This shows that the two plants almost have equal moisture contents. The moisture contents in different parts of *E. prostrata* were distributed as; seeds > leaves > stem > whole plant > roots. Exactly same distribution was found in the *E. granulata*. The higher and lower ranges were 13.4% ± 0.13 for seeds and 4.9% ± 0.22 for roots (Fig. 13) in present. This range was 12.2 ± 0.379 and 4.7% ± 0.25 in case of *E. granulata* (Fig. 4). Since both plants belongs to same genus, grows in similar habitat, they developed similar adaptation to fulfill water needs. This supports the present findings and suggests that seeds need more quantity of water to overcome the dry season.

Fig. 11 shows 12.7% ± 0.15 ash contents in *E. prostrata* whole plant. However, ash contents of *E. granulata* were 11.57% ± 0.41. This reveals that *E. prostrata* contained 8.9% more ash than found in *E. granulata*. As the ash in a plant represent the inorganic mineral (Aberoumand, 2010; Folorunso &Modupe, 2007; Yusuf *et al*., 2007) hence the present plant seem with more minerals than previous. Comparing the mineral composition of the two plants (Tables 8 and 9); *E. prostrata* contained more Fe, Mn, Zn, Mo, P, S, N and Se than found in *E. granulata*. This supports our findings of high minerals in *E. prostrata*. Vertical distribution of ash in present plant parts shows the order of; stem > leaves > whole plant > roots > seeds (Fig. 14). However this order was a little changed in *E. granulata* i.e. stem > whole plant > leaves > roots > seeds (Fig. 5). This suggests that in case in *E. prostrata* the need of leaves for minerals is more than the overall need of the plant. The rest of the parts had almost similar needs and identical ash contents.

E. granulata whole plant had 22.6% more fats i.e. 4.2% ± 0.3 (Fig. 6) compared to *E. prostata* contained 3.25% ± 0.07of fats (Fig. 15) while. Comparing the lipid contents in plant parts of the two plant, seed of both plants contained large quantities of lipids. In case of *E. prostata* 21.4% ± 0.18 of the total fats were in seeds (6.6 times of whole plant) while *E. granulata* had 19.9% ± 0.351(4.7 time of whole plant). Seeds need a lot of water and stored energy especially in plant of dry habitats to overcome the extreme deficiency periods and support germinating ovules. High levels of lipids and moistures in seeds are also reported by other researchers as well (Yusuf *et al*., 2007). In both studied plants the levels of lipids in stem, leaves and roots are not enough to be discussed further. The present findings revealed that the seeds of two investigated plants are equally good source of lipids.

The study further revealed that *E. prostrata* contained slightly more dietary fibers (20% ± 0.49) than found in *E. granulata* (18.3% ± 0.68). Evaluating plant parts showed that of the total fibers found in plant, major contribution was made by the roots. Roots of *E. prostrata* contained more fibers (29.4% ± 0.31) as compared to *E. granulata* (27.8% ± 0.61). The function of fibers is to retain water. comparing moisture contents of two plants it was found that roots, leaves and seeds of *E. prostrata* contained more moistures than *E. granulata* (Figs. 4 and 13). Proportionately roots and leaves of *E. prostrata* contained more moisture (Fig. 13) compared to that of *E. granulata* (Fig. 4). That strengthened our findings. High moisture and small fiber contents in seeds contradict the above claim but hard integument of seeds help seeds to retain a lot of moistures in its cellular portion. The overall comparison shows that *E. prostrata* is a better source of fiber than *E. granulata*.

Analysis for dietary protein shows that 18.3% ± 0.18 of it were present in the whole plant (Fig. 17). Seeds contained the highest amount of 23.6% ± 0.11, followed by leaves with 21.2% ± 0.06. Least of the proteins were recorded for stem (16.4% ± 0.03). In previous experiment *E. granulata* was noted with 17.5% ± 0.52 of protein in whole plant (Fig. 5). Similar to the present plant it had high proteins in seeds (22.4% ± 0.379), followed by leaves (21.2% ± 0.06). As noted for moisture, ash, and lipid contents of the two plants, protein had also similar distribution in parts of two plants. Although, *E. prostrata* looks more nutritious compared to *E. granulata*, but the ratio in quantity is not

too much. This suggests that both plants could be equally beneficial in providing proteins and essential amino acids.

Unlike other nutrients, the carbohydrate contents found in *E. prostrata* were less than *E. granulata*. Whole plant of *E. prostrata* showed 32.3% ± 0.2 of carbohydrates however this amount was 39.9% ± 1.32 in above plant. Ratio of 32.3 to 39.9 in the two plants suggests a 32.4% less amount of carbohydrates in *E. prostrata*. As carbohydrates are quick source of energy, *E. prostrtata* seems to be a poor source of quick energy. Stem was noted for poor amount of proteins above but in case of carbohydrates the stem was found as a rich source (35.9% ± 0.47) compared to other parts (Fig. 18). In both plants least of the carbohydrates were found in seeds Distribution of carbohydrates in *Euphorbia prostrata* parts was found as; stem > leaves > whole plant > roots > seeds (Fig. 9). There was very little difference amongst the highest (35.9 ± 0.47%), and the lowest noted value (31.1 ± 0.21%). The amount of carbohydrates found in the two plants, could be a possible source of carbohydrate derivatives that might have therapeutic potential.

All the pH values noted for plant and plant parts ranges on the acidic side of pH levels i.e. they were on the lower side of the 7. As majority of metabolic cycles during photosynthesis and respiration results in some intermediate acidic molecules this is why more acidity prevails in plants. This suggests that dry powdered plant material contained good number of metabolites. The pH of whole plant was 4.6 with higher (5.4) in stem and the lowest for leaves (4.1). Comparing these values with values of *E. granulata*, small differences were noted in whole plant plant parts. The previous plant was found less acidic with relatively high pH value (4.9) than the present plant (4.6). Although, the differences were small but percentage wise, it is 6.1%. Small differences in plant pH can results in big differences plants biochemical constituents (Krishnamurthy & Sarala, 2010). Base on the differences in acidity of the two plants it can be said that *E. prostrata* is likely to contain more acidic metabolites than in *E. granulata*.

When total soluble solid (TSS) were compared in both plants, it was almost equal (Figs. 11, 20). Both plants had high level TSS (0.5%) in roots, followed by whole plant (0.3%). The stem and leaves contained equal amount of TSS (0.2%). the least amount

was noted for seeds (0.1%). Similarity in TSS is another evidence of biochemical and physiological similarity of the two plants.

Different food nutrients provide different calories. More calories are produced with fats, 1 gram of which gives 9 calories. Carbohydrates and proteins provide 4 calories with 1 gram of each. Living organisms stores extra energy in the form of fats. The foods rich in fats provide high energy to the body (Blake, 2012). Plants contain different nutrients in different proportion. The present plant contained only 3.25% of lipids. This suggests that the whole plant is not a rich source to provide calories. Protein contents were 18.3% and carbohydrates 32.3%. This suggests that most of the calories found in *E. prostrata* are from NFE and protein. Base on the proximate composition of the plant energy of the two plants were calculated. Figure 21 shows that 261.01 kilo calories can be obtained from 100 gram of plant. Most of these calories (158.6) are from NFE. Protein gives 73.16 while lipids contribute the least (29.25). Considering the case of *E. granulata*, it was observed that it contribute a little more than the present plant i.e. 276.4 K.C. Lipids, protein and NFE had similar contribution as with the present plant. It is concluded that these two plants have almost identical chemical nature as they belong to same genus. People used them for same purpose and they have almost similar proximate composition

2. Phytochemical analysis

Euphorbia granulata Forssk

The preliminary results for different phytochemical are listed in Table 4. The quantitative composition of plant and plant parts are summarized in Table 6.

On account of weak chemical reaction the color produced for terpenes, terpenoids, anthraquinones, anthocyanins and were not considered for quantification. Total phenolics, alkaloids, flavonoids, tannins, glycosides and saponins were detected and then confirmed with strong colored reactions. These were then quantified. Whole plant contained comparitivly more total phenolics (0.8 ± 0.025 mg/gm) than other phytochemicals. Alkaloid and flavoids were the second important constituents, which were present in equal amounts. Flavonoids were 0.7 ± 0.02 mg /gm and alkaloids 0.7 ±

0.006 mg / gm. Tannins were 0.6 ± 0.025 mg / gm. Both glycosides (0.2 ± 0.012) and saponins were (0.2 ± 0.0 mg/gm) found in small but in equal amounts. Vertical distribution of total phenolics in plant parts reveals the high concentration was in the leaves (1.1 ±0.012 mg/gm). Herbal medicines contain unique ingredients. They differ from plant to plant and therefore the therapeutic impact also differs (Roopashree *et al*., 2008). Metabolically leaf is the most active part of the plant. The high quantity of phenolics in leaves suggest that they are likely to be synthesized in leaves. Herbivores mostly use leaves for food purpose. The large amount of them in leaves might be an adaptation to avoid being eaten by animals. Also the high quantities of these compounds in leaves suggest the high therapeutic values of leaves. Small amount of phenolics was found in roots. High concentration of flavonoids (0.9 ± 0.0) and alkaloids (0.8 ± 0.015) was also found in roots. Researchers refer the antibacterial and antioxidant effects of plants to the presence of different forms of phenolic compounds especially flavonoids (Shan *et al*., 2007; Dorman & Deans. 2000). The 0.8 mg/g total phenolic compounds and 0.7 mg/g flavonoids found in *E. granulata* whole plant may have contributed to the antibacterial and antioxidant effects of this plant. Flavonoids are also considered as hepato-protective and cardioprotective (Yasmin *et al*., 2011). The quantity found in the present two plants may have contributed to these protective effects (Ramkumar *et al*., 2007). Tannins were absent in roots, but found in large proportion in leaves (0.6 ± 0.01 mg/gm). This quantity was three times greater than the quantitity found in whole plant (0.2 mg/gm). Tannin is a good antioxidant and its presence in plant may be of use in free radical scavenging activity (Patel *et al*., 2010; Hamid *et al*., 2011). Tannins are thought to speed up wound healing and mitigate mucus membrane inflammation (Obianime & Uche. 2008). These days the anticancer potential of fruits and vegitables is a hot topic for discussion. The active ingredients presents in medicinal plants is thought to control growth of cancerous cells with different controlling mechanisms. They have also proved to be helpful in preventing and treating other diseases in human. Studies have shown their usefulness in physiological disorders like inflammation, diabetes, psychotic diseases, high blood pressure and infections. Researchers often link the antioxidant potential of plant constituents to their anticancer activity (Fayed *et al*., 2009). On this base the

presence of tannins in *E. granulata* can be linked to unknown beneficial effects. Glycosides in stem (0.6 ± 0.025) and leaves (0.7 ± 0.035) were present in almost equal amount however roots had little amount (0.3 ± 0.173). Many reports have linked glycosides with insect repulsive property of plants (Ghafour *et al*., 2010; Daniel & Dishi, 2011). In a separate experiment the plant was tested for insecticidal activity but no effect was noted (Tables 65-67). Although, 0.6 mg/gm of glycosides was found in *E. granulata* but this quantity might not be enough for insect repulsion. Different structural forms of glycosides are also considered to be responsible for antifungal effect (Chew *et al*., 2011). Antifungal effect found in *E. granulata* may be due to the presence of some unique glycosides. Saponins were absent both in stem and roots but were found in very small amount (0.2 ± 0.025) in whole plant and leaves.

Different classes of these secondary metabolites have different impacts e.g. flavonoids are cardiovascular protectors, antiviral, anti-inflammatory and antiallergic. Another class named as terpenoids presents in most vegetables and is called as carotenoids have gain attention due to its high antioxidant properties. They also have role in immune response and prevent macular degeneration (Premier, 2002). Although, not tested for all these physiological problems but it seems that the present combination of these phytochemicals in *E. granulata* may have positive impacts in these problems.

***Euphorbia prostrata* Aiton**

The results for screening different phytochemicals in *Euphorbia prostrata* are shown in Table 5. Terpenes, terpenoids, phlobatannins, anthocyanins and coumarins did not produce color reaction. The phytochmicals that give strong chemical reaction were then quantified. Like *E. granulata,* total phenolics, flavonoids, alkaloids, tannins, glycosides and saponins were strongly detected and quantified. The quantity found for each phytochemical are shown in Table 7.

When different phytochemicals were compared it was seen that total phenolics were 1.0 (± 0.153) mg/gm. This quantity was 20% more compared to *E. granulata*. Glycosides 0.9 ± 0.025 mg/gm were second in quantity. This concentration was 33.3% higher when compared to *E. granulata*. The glycosides were the third major constituent in *E. granulata* in terms of quantity. The amount of alkaloids (0.6 ± 0.015) and

flavonoids (0.5 ± 0.03) was less compared to found in *E. granulata*. Tannins (0.3 ± 0.015) and saponins (0.2 ± 0.006) were present in small amount. Surprisingly saponins in both plants were found in equal amount (2.0 ±0.006 mg/gm). All the six phytochemicals were mostly distributed in the leaves. Out of them, total phenolics and glycosides had equal concentrations in both the plants, i.e. 1.2 mg/gm. Roots had second high amount of alkaloids (0.9 ± 0.025). Similar to phenolics and glycosides, the leaves had equal amounts of flavonoids (0.7 ± 0.025) and tannins (0.7 ± 0.025). The amount of saponins was the least in the leaves. The overall results reveal that whole plant is *E. prostrata* richer in different phytochemicals compared to *E. granulata*. This may be correlates with high antioxidant and antibacterial effects found in *E. prostrata* compared to *E. granulata*. The small differences in physiological impacts of the two plants seem the direct effect of the different combinations of these phytochemicals (Roopashree *et al*., 2008).

Very small variation was noted in phytochemical composition between the two plants. That is why most of the medicinal properties and physiological impacts resulted with the two plants extracts were also identical.

3. Mineral Composition

***Euphorbia granulata* Forssk**

Mineral composition of soil was analyzed to check for toxic concentration and extraction efficacy of the plant. The minerals composition in soil is presented in Table 8. The quantitative presences of different mineral in *Euphorbia granulata* are summarized in Table 9. In soil the high to low concentration order was S > Ca > P > Fe > K > Na > Mg > Cl > Mn > Zn > Cu > Mo > Se >N. In whole plant these 14 elements were present as S > K > Mg > Na > Ca > P > Fe > Mn > Zn > Se > N > Mo > Cu = Cl. The difference in the two orders of the elements suggests that plants are highly selective in absorbing elements from soil. All the elements in the soil were found within the permissible limits (Ramamurthy & Kannan, 2009). Mineral may be either requiring in small (micro-minerals) or large amount (macro-minerals).

There is no precise definition for trace or micro-mineral. However, micro-mineral are those elements that constitutes <0.01% of the total body weight (Gropper *et al*., 2009). Optimum level of mineral (both macro and micro) nutrient is essential for good

health. Deviation from optimum limits growth of plants and produces physiological problems in man. Various elements play different roles in plants metabolism. The present study analyzed and compared fourteen mineral elements in whole plant and parts of *Euphorbia granulata*.

Iron is an important trace element. It is part of hemoglobin. Iron play role in the metabolism of lipids, carbohydrates and protein. Deficiency of iron can lead to anemia (Khan *et al*., 2008; Shad *et al*., 2002; Krishnamurthy & Sarala, 2010). Higher concentration was noted for leaves (919 ± 2.08 ppm) while low for seeds (105 ± 0.75 ppm). The higher concentration in leaves suggest the high needs of leaves for its high metabolic activities. As seeds remain dormant for long time they may needs small amount of iron to meet the need. In whole plant iron concentration was 803.3 ± 4.4 ppm. The permissible limit (Table 11) of iron for edible plants is 20 ppm (Mtunzi *et al*., 2012). This shows a concentration for high than permissible limit but *E. granulata* is a medicinal plant and is used in small amount. Iron is a micro-mineral and is needed for good health (Gropper *et al*., 2009). For a healthy male of age between 19 to 50 years, the recommended daily allowances (RDA) are 8 mg/day (Table 11). As iron is part of hemoglobin and many metabolic enzymes this suggests that *Euphorbia granulata* might be a good source of Iron.

Table 9 shows that high Manganese accumulation (68.9 ± 0.8 ppm) occurred in leaves. Little variation was noted in roots, stem and leaves. However, seeds contained 4 times less manganese (18.53 ± 0.088 ppm) than the leaves. Manganese is needed for proper functioning of many enzymes, including transferases, hydrolases, oxidoreductases, ligases, and lyases (Gropper *et al*., 2009). It is important for the development of bones, fats and carbohydrates metabolism. Mg deficiency can leads to myocardial infarction, formation of defective cartilages in infants, immunodeficiency and rheumatoid arthritis (Shad *et al*., 2002; Mtunzi *et al*., 2012; Khan *et al*., 2008). For ingestion the permissible range for Mn is only 2 ppm for edible plants (Table 11). The plant seems rich with Mn in it but the amount which is usually ingested small and will not be harmful. Furthermore the element is essential to be taken in diet. The RDA for Mn is 2.3 mg (Table 11). The

concentration found in *Euphorbia granulata* and the daily need of the body suggests that the use of plant for medicinal purpose can be a good source of this micro-mineral.

Zinc is another important micro-mineral. Zinc is needed for normal insulin secretion, wound healing, normal growth and development, brain health and behavioral development (Khan *et al*., 2008). Distribution order in plant parts was as; roots > whole plant > stem > leaves > seeds (Table 9). High amount in roots indicates the high retention of roots for Zn for supplying it to the aerial parts. The permissible limit for edible plants is 27.4 ppm, while Zn found in whole plant was 47.1 ± 0.09 ppm (Tables 9, 11). Roots contained 52.7 ± 0.12 ppm while seeds 19.45 ± 0.058 ppm. The present concentration in the whole plant is almost double of the permissible limit. Based on the need of the body for Zn the suggested RDA is 11.0 mg (Table 11). The use of *Euphorbia granulata* for medicinal purpose might help providing the mineral in sufficient amount to fulfill the patient need for Zn.

Copper is also an essential micro-mineral. It may be toxic if taken in large amount. The permissible amount to be present in edible plants is 3 ppm (Table 5). In the present study copper was not detected whole plant while roots were noted with 1.9 ± 0.02 ppm and seeds with 0.66 ± 0.017 ppm (Table 9). This reduces the chances of copper toxicity with consumption of plant. Furthermore, ceruloplasmin, superoxide dismutase, cytochrome c oxidase, amine oxidases, lysyl oxidase and many more enzymes needs copper as a cofactor (Gropper *et al*., 2009). Copper is required for absorption and incorporation of iron in to hemoglobin, RBCs formation, mitochondrial activities and RNAs the deficiency leads to anemia, fragility of blood vessels and bone cortices (Shad *et al*., 2002; Krishnamurthy & Sarala, 2010). Sufficient amount of the copper should be present in the diet to fulfill the daily requirements. The suggested RDA is 0.9 mg (Table 11). The amount found in *Euphorbia granulata* suggests that this plant is not a good source of copper however the amount found in roots and seeds will contribute to the overall need of the body.

Molybdenum is essential part of body enzymes like xanthine oxidase, nitrate reductase and sulfite oxidase (essential enzymes for electron transport). Molybdenum within permissible limits (0.02 ppm) help in copper utilization but high intake produces

copper deficiency which leads to microcytic anemia (Erdman *et al*., 2012; Gropper *et al*., 2009). To meet the need of the body metabolic activities the suggested RDA is 45µg for molybdenum (Table 11). The element found in *Euphorbia granulata* was distributed in plant parts in order of, roots > leaves > whole plant > stem > seeds (Table 9). Mo concentration in whole plant was 1.9 ± 0.03 ppm, while permissible concentration in edible plants is 0.02 ppm (Tables 9, 11). Higher concentration was noted for roots (2.3 ± 0.03 ppm) while not detected in seeds. The high concentration noted for Mo in plant and plant parts apparently looks high but the plant is not an edible one and used in small amount. The overall amount found in plant suggests that its ingestion will help in providing molybdenum to the daily need of consumer.

Selenium, a micro-mineral, has its role in preventing liver cell necrosis and other beneficial effects in human and animal health. It is a prosthetic group for the enzyme glutathione peroxidase which is involved in reducing hydrogen peroxide to water and hence helpful anti-oxidant role (Erdman *et al*., 2012; Gropper *et al*., 2009). Table 9 shows that *Euphorbia granulata* whole plant contain (34.46 ± 0.08 ppm), of which major portion is located in roots (89.8 ± 0.04 ppm). Seeds contained only 2.78 ± 0.043 ppm. As the body need very small amount of selenium (RDA 55µg / day), the amount present in plant suggest that it will benefit the health of consuming patient.

Macro-minerals are elements that at least constitute 0.01% of the total body weight (Gropper *et al*., 2009). Calcium is a macro-mineral which is vital for bone and teeth health. It is also needed for cardiac muscles and nerve impulses, blood and milk clotting (Krishnamurthy & Sarala, 2010; Hussain *et al*., 2011; Mtunzi *et al*., 2012). It is highly needed in the body for the daily turnover of bone. For a person having an age between 19 and 50 years the suggested adequate intake (AI) is 1000 mg for (Gropper *et al*., 2009). *Euphorbia granulata* was found with good quantity of calcium. Higher amount was found in leaves compared to concentration found in whole plant and the rest of the plant parts. High amount of calcium is necessary for high metabolic activity in meristematic regions of plants because calcium is essential for cell division (Krishnamurthy & Sarala, 2010). Calcium concentration in whole plant was 3007 ± 14.5

ppm. The high concentration noted for Ca in plant and plant parts may be good for the consumer health.

Phosphorus is also an essential part of bones, it is part of phospholipids (an essential cell membrane component), constitute part of ATP (an energy rich molecules) and nucleic acids (Shad *et al*., 2002; Yusuf *et al*., 2007). The results shown in Table 9 revealed high phosphorus contents in *Euphorbia granulata* especially in leaves. Growing cells needs a lot of energy shifts and hence a lot of energy rich ATP molecules. Similarly, during cells multiplication more and more nucleic acids are synthesized. This necessitates the availability of phosphorus; therefore, higher quantity of phosphorus is necessary for growing parts of the plants. Phosphorus concentration in whole plant was 1638 ± 6.0 ppm, while in roots it was 1998 ± 6.0 ppm. Almost matched quantity of phosphorus with leaves, found in seeds suggests that high levels of phosphorus will be needed at the time of seed germination. Based on the high needs of phosphorus the body metabolic activities high RDA (700 mg/day) is suggested by NSA, (2005). As macro-nutrients are required in large amount permissible concentration in edible plants is not specified (Tables 9, 11).

Almost 55 to 60% of total magnesium is associated with bones and the rest is tied up in muscles and extracellular fluids. It is needed for enzymes of respiration (glycolysis and TCA) especially ATP or ADP and associated enzymes (Gropper *et al*., 2009). Its deficiency causes muscle irritability and convulsion (Yusuf *et al*., 2007; Krishnamurthy & Sarala, 2010). For adult male the RDA is 400-420 mg (Table 11). Table 9 shows that leave of *Euphorbia granulata* are rich in Mg, which can be an efficient source of magnesium. Mg concentration in whole plant was 4883 ±20.27, while it was 5265 ± 7.63 ppm in leaves. The least Mg quantity in seeds (2198 ±6.0 ppm) indicates the reduced metabolic activities of seeds. As Mg is required in large amount the permissible concentration in edible plants is not specified (Tables 9, 11).

Sulfur is part of acetyl co-A, amino acids, vitamins and lipids like mucoitin and chondriotin (Erdman *et al*., 2012; Gropper *et al*., 2009). Distribution in plant parts was as; roots > leaves > whole plant > stem > seeds (Table 9). High level in roots (35633 ± 60.09ppm) might be the retention by the roots exudates. Sulfur concentration in whole plant was 31183 ± 60.09 ppm while seeds contained least amount of 21070 ± 17.32 ppm.

Both permissible concentrations in edible plants and RDA for human are not specified (Tables 9, 11). The high concentration noted for sulfur in plant and plant parts is good which reflect that sulfur containing amino acids, lipid and vitamins are in sufficient amount and is good for the patient health.

Nitrogen is an essential element and part of protein, porphyrins, lipid, purines, pyrimidine and vitamins. The nitrogen contents of a plant reflect the protein contents in the plant which is already discussed in protein portion. For adult male the required RDA is 56.0 gm proteins/day (Table 11).

Potassium is the major extracellular cation widely present in body fluids and tissues. It is involved in muscular activities, acid base balance, and neuromuscular activities. It is parts of certain metabolic enzymes like pyruvate kinase and has important role in cardiac function (Erdman *et al*., 2012; Gropper *et al*., 2009; Krishnamurthy & Sarala, 2010; Kawo *et al*., 2009). Potassium concentration in different parts of *Euphorbia granulata* was; stem > whole plant > leaves > roots > seeds (Table 9). Similar results of potassium distribution in plant parts were also reported by Shad *et al*, (2002). Potassium concentration in whole plant was 12533 ± 109.3 ppm. Highest concentration was noted for stem (12800 ±15.275) and the lowest for seeds (1332 ±4.41). High concentration in the plant may help restoring the electrolyte balance in patient of diarrhea. The high RDA for potassium (4700 mg) indicates the need of a continuous supply. While taking plant by a person, some parts of the needed potassium will be obtained from this plant.

Sodium is cation and macro-mineral, which is found in extracellular body fluids. Its major role is electrolyte balance (Erdman *et al*., 2012; Gropper *et al*., 2009; Krishnamurthy & Sarala, 2010). Sodium is also needed in high amount by the human body (RDA 1300-1500 mg daily). Table 9 shows 19050 ± 86.60 ppm in whole plant, major portion of which is distributed into the roots (4261 ± 4.41 ppm). Only 470 ± 5.23 ppm was recorded for seeds. This shows that plant roots contained 9 times more sodium than the seeds. As roots are in direct contact with soil and soil is the only source of electrolytes for plants the large proportion in roots might accumulation due to this direct contact with soil. Plant most often contain variable amount of sodium but the daily requirements of human are most often met from table salt.

Chlorine is important for production of HCl in stomach and chloride shift during respiration (Erdman *et al*., 2012; Gropper *et al*., 2009). Chloride concentration in whole plant was below detecting limit. It was detected in roots only (1.98 ±0.06). Plants are not good source of chlorine while the human need it in large amount (RDA 2300 mg/day). To fulfill the requirements just like sodium the human get the additional chloride from table salts (NaCl).

Most of the essential mineral elements are required in minute amount (iron, manganese, zinc, selenium and molybdenum etc.) and the plant has the potential to supply these elements at therapeutic dose. Analysis of *Euphorbia granulata* revealed only copper and Chlorides are present in small amount. While the remaining minerals are present in higher concentrations and that might seems as a good source of these minerals.

***Euphorbia prostrata* Aiton**

Mineral composition of the soil and the permissible concentration in soil are presented in Table 8. The levels of these minerals in whole plant and their distribution in different plant parts of *E. prostrata* are tabulated in Table 10. The purpose of checking the levels of mineral elements in soil, as well as in plants and its parts was to check the plant need, its extraction efficiency and the possible toxic level in the soil.

The concentration in whole plant was in order of S > Ca > P > Fe > K > Na > Mg > Cl > Mn > Zn > Cu > Mo > Se. Comparing the values in plant (Table 10) to permissible levels (Table 11), shows optimal levels of all these elements in plant.

More iron (1112.6 ± 9.93) contents were noted in *E. prostrata* compared to that found in *E. granulata* (803.3 ± 4.4). This was 38.5% high quantity than the value in *E. granulata*. This suggests high value of the *E. prostrata* compared to *E. granulata*, in terms of supplementing iron to the consumer. As mentioned for in previous study, this quantity is above the permissible limits of 20 ppm (Table 11) but that amount is for edible plants only. A proportional increase was also found in all parts of the present plant. Like the previous plant high quantity of iron was distributed to leaves (1395 ±7.6). In relation to whole plant the quantity in leaves was 20.3% higher. This suggest that taking leaves only will be more beneficial in providing iron to the body compared to whole plant. Furthermore the anemic patients will be more benefited from *E. prostrata* than *E.*

granulata and in turn more benefited from leaves than other parts of the plant or whole plant. Representative soil sample had 23233 ± 504.4 mg/gm of iron. The amount found in *E. prostrata* was 1112.6 ± 9.93 mg / gm. The amount in plant was only 4.8% of the amount in soil. This suggests that absorption by plant was not affected by the high quantity in soil but was absorbed in quantity based on the need of plant.

Analysis of Mn in soil (516 ± 12.01) and *E. prostrata* (46.4 ±0.03) revealed only 8.9% in plant body. Unlike iron, the amount of Mn in *E. prostrata* (46.4 ±0.03) was less compared to *E. granulata* (65.1 ± 0.38). Permissible limit in edible plant is 2 ppm (Mtunzi *et al*., 2012). This suggests that *E. prostrata* contained higher amount than required in edible plants but so far permissible in medicinal plants is not set. At present concentration the plant is good source of Mn and will easily supply the RDA recommended by NAS (2005). The quantity found in different parts a show that leaves with 51.3 ±0.43 mg / gm could be a more efficient source of Mn compared to other parts. Overall, *E. granulata* contained comparatively small quantity than *E. granulata* but even then may be an efficient source of Mn.

In term of therapeutic amount of zinc present in the two plants, *E. prostrata* (72.5 ±0.73 mg/gm) is better source compared to *E. granulata* (Tables 9 & 10). The amount of Zn found in different parts of the plant revealed that more zinc was present roots than raised to aerial parts. Compared to the amount in whole plant, the roots contained 15.3% more zinc (85.8 ±0.6 mg/gm). This suggests that in case of Zn deficiency roots could be an efficient source. As zinc helps in wound healing and proper insulin secretion the plant could be able to produce positive results in diabetic patients. The high Zn quantity in *E. prostrata* can be correlated the better results with *E. prostrata* in diabetic rabbits than in *E. granulata* (212 ± 13.864 to 205 ± 13.58) in Tables 20 and 21. The distributions in different parts of the plant showed complete harmony with *E. granulata*. This could due to similarity in biochemistry of the two plants.

In spite of high importance of copper in iron metabolism and red blood cells formation the whole plant had not enough of it to be detected. Lack of Cu was also noted for *E. granulata*. Of the four plant parts Cu was present in small quantity in roots (1.13 ±0.06), followed by 1.33 ±0.03 and then seeds 3.3 ±0.06 mg/gm. Stem did not had Cu.

This suggests that in whole plant powder the main portion was from stem. This high ratio of stem, in whole plant powder tends the level of Cu not to be detected in whole plant. Comparing the percent distribution of Cu in plant parts of *E. prostrata* with *E. granulata* show that, in first case more Cu was present in leaves (3.3 ±0.06) while in second case more or Cu was present in roots (1.9 ± 0.02). The small amount of Cu in both plants may also be affected with its small concentration (2.7 ± 0.06) found in soil. Table 11 shows that 3 ppm is the permissible limit for Cu in edible plants. It is needed in very small amount of only 0.9 mg/gm. The present concentration in parts of both plants is well below this amount in whole plant. The overall concentration in different parts of the two plants suggests that the need of Cu "if any" can be meet from leaves of *E. prostrata*.

The daily need of molybdenum for proper physiological functions is only 0.045 mg/gm. The present plant contained 2.0 ± 0.05 mg/gm. This amount was only 5.3% high compared to found in *E. granulata*. This suggests and equal need of the two plants for Mo. In case of *E. granulata* the high amount was in roots (2.3 ± 0.03) while in present case the Mo rich part was leaves. Surprisingly the amount of Mo in roots and leaves of the two plants were inverse to each other i.e. 2.3 ± 0.03 mg was in roots of *E. granulata* but this much amount (2.3 ±0.019) was in leaves of *E. prostrata*. Similar concentration of 2 ±0.04 in roots of *E. prostrata* was recorded in leaves of *E. granulata*. In both plants equal amount of Mo was found in stem (1.49 ±0.2 and 1.5 ± 0.29) and BDL in seeds. Out of discussed mineral elements, so far, Mo came out as the only element that was distributed differently in different parts of the two plants. For Mo the permissible limit in soil is 4 ppm (Chesworth, 2008). Representative soil sample had 1.5 ppm (Table 8). As already pointed out, Mo is essential for ETC, the results reveal that metabolically active parts (roots and leaves) had the highest amount. The present concentration in plant is enough to provide the quantity needed by the consumer body.

E. prostrata had 3017 ±20.27 mg/gm of calcium. This amount was equal to Ca in *E. granulata* (31183 ± 60.09). These quantities indicate that both the plants have identical needs for calcium. In case of *E. prostrata* more Ca was accumulated in leaves (4480 ±51.31). Stem and seeds had almost equal amounts i.e. 3453 ±14.53 and 3381 ±8.02 respectively. Roots had the smallest quantity (2516 ±31.8). Comparing these distributions

with *E. granulata* shows that the richest parts were roots (36157 ± 34.8) and leaves (35633 ± 60.09) while the poor part the seeds (21070 ± 17.32). Considering the overall picture it appears that there is very little difference in the two plants. As the daily need for calcium is 1000 mg (Table 11), the available calcium in the two plants can be a suitable calcium supplement. Furthermore comparing the levels of Ca in the plant and that in soil (33200 ± 86.8) shows that, both the plants are equally efficient in obtaining calcium from the soil.

Although both calcium and phosphorus have role in bone and teeth but unlike calcium, phosphorus is needed in comparatively small quantity. For good health 700 mg of phosphorus is needed daily (Table 11). The concentration in *E. prostrata* whole plant was 1844 ±21.85 mg/gm. This amount was comparatively more than noted for *E. granulata*. This suggests that *E. prostrata* might contribute more to the bone health. Further analysis revealed that leaves contained more P (2203 ±8.81 mg/gm) than the amount in whole plant. The second highest quantity was noted in seeds (1855 ±7.6) and the least amount in roots (1310 ±11.5). Similar distribution trend of P in different plants was also noted for *E. granulata*. Although the permissible limit in soil is not specified however the representative soil sample was rich in P (27116 ± 116.66) to full fill the need of the plants.

After sulfur (33550 ±275.38) and potassium (9100 ±50), magnesium was the third most abundant element (4400 ±132.29) in the plant body (Table 10). Soil sample had 5433 ± 60.09 mg/gm against the permissible limit 9000 ppm. Like calcium the absorbance of Mg was also very efficient compared to its concentration in the soil sample. The present plant have comparatively small amount of Mg than *E. granulata* had (4883 ± 20.27). Out of the four plant parts leaves were enriched with 5100 ± 57.73 mg/gm concentration of Mg. seeds had the least of Mg (3255 ± 5.7). Similar pattern of Mg was also found in *E. granulata*. The high levels in leaves may be due to the high metabolic need of the leaves. The present concentration in the two plants is well above the RDA (400-420) recommended by NAS, (2005).

Off all the minerals analyzed in both the plants, sulfur was found in the ever highest concentration (33550 ± 275.4). Representative soil sample had also the highest S

(45633 ± 88.19) than other elements. Out of this total, 37500 ± 288 mg/gm was found in the roots. Stem and seeds had almost equal and the lower levels of Mg i.e. 24933 ± 233.3 and 2346 ± 4.9 respectively. The results seem that high concentration in plant might was likely to be influenced by the large quantity in soil. Sulfur is an essential part of many vitamins, amino acids, lipids and acetyl co-A. The large amount in the two plants suggests that both of them are rich in sulfur containing vitamins and other important nutritional components.

Major of portion of nitrogen in the living organisms contributes to the protein contents. The present whole plant contained 2.92 ± 0.031 ppm. This amount was relatively more than that in *E. granulata* (2.80 ± 0.02 ppm). Analysis of plant parts shows that seeds of *E. prostrata* had high amount of nitrogen (3.77 ± 0.017 ppm). This suggests that seeds contained more proteins than other parts. During proximate analysis the seeds of *E. prostrata* were found rich in proteins (Fig. 14). The present data shows the order of nitrogen in the plant as seeds > leaves > whole plant > roots > stem. Exactly same order of increasing protein was calculated for the plant. The findings support the presence of good amount of proteins in the two plants.

The second abundant element was potassium (9100 ± 50) in *E. prostrata*. Although not assimilated into organic matter the proper amount is essential for metabolic activities of living cells. The stem had the highest K contents of 12400 ± 208.1 ppm, followed by leaves (6983 ± 44.1) and roots (465 ± 8.66 ppm). This represents 26.7 times more K in stem than in roots. In the present case 37.7% less potassium was noted compared to *E. granulata*. Potassium is important cellular cation. Recommended RDA is 4700 mg (Table 11). The high quantity in plant indicates that both plants could be a rich source of K.

Sodium has no specific role in plants except influencing carbon dioxide concentration in C4 plants and keeping high osmotic pressure and turgidity especially in stem. Therefore plant scientists don’t classify Na as essential mineral for plant. Its presence in plants is ubiquitous. *E. prostrata* was noted with 1110 ± 22.68 ppm of sodium. Furthermore, roots were found with 2610 ± 23.1 and stem with 1377 ± 14.5. Plants are not considered as important source of sodium in human, because table salts

fulfill these requirements. The sodium in present plant can be of possible benefits for herbivores.

Like *E. granulata*, *E. prostrata* also had undetected level of chlorides.

The role of selenium in plant body is also not clear. However, Se is a micro-mineral for human its presence in plant can be a source for him. The present plant contained 74.7 ± 1.12 ppm of it. Major portion of it was noted in roots i.e. 144 ± 0.66 ppm while seeds had small amount of 27.1 ± 0.11ppm. Selenium is an essential part of glutathione that play important role in antioxidant acitivity. Therefore Se in plant could be a possible source for antioxidant activity in human body.

Overall comparison of the mineral elements in the two plants revealed that they are equally important for the two and might be important source of all minerals.

Table 1. Detection results for different nutrients of *Euphorbia granulata*.

Test	AC	RS	MS	KS	AS	Protein	A. acid
Detection	p	p	P	p	p	p	p
Confirm	nd	p	nd	p	nd	p	nd

Table 2. Detection results for different nutrients of *Euphorbia prostrata.*

Test	AC	RS	MS	KS	AS	Protein	A. acid
Detection	p	p	p	p	p	p	p
Confirm	nd	p	nd	p	nd	p	nd

AC = All carbohydrates, RS = Reducing sugar, MS = Monosaccharides, KS = Ketose sugar,
AS = Aldose sugar, p = present, nd = not done

Table 3. Classes of food in term of nutritional values.

Type of Nutrients	Food values in term of calories (grams for fibers) by Rinzler (2009)		
	Low value	Moderate value	High value
Protein	< 5%cal	5-20% cal	> 20%cal
Lipids	< 30% cal	30-50% cal	> 50% cal
Carbohydrates	< 20% cal	20-60% cal	> 60% cal
Dietary fibers	< 1gm/serving	1-5gms/serving	>2gms/serving
Whole food	<50cal/100gms/serving	50-250cal/serving	>250cal/serving

Table 4. Detection of phytochemicals in whole plant and plant parts of *E. granulata.*

Test	AKs	TPn	TPd	TNs	SPn	GSd	ANq	PHn	FNd	PBt	ANc	CRn	STd
WP	p	A	A	p	p	p	p	p	p	A	A	A	A
RTs	p	A	nd	A	A	p	nd	p	p	nd	nd	nd	nd
ST	p	nd	nd	p	A	p	nd	p	p	nd	nd	nd	nd
LVs	p	nd	nd	p	p	p	nd	p	p	nd	nd	nd	nd

Table 5. Detection of phytochemicals in whole plant and plant parts of *E. prostrata.*

Test	AKs	TPn	TPd	TNs	SPn	GSd	ANq	PHn	FNd	PBt	ANc	CRn	STd
WP	p	A	A	p	p	p	p	p	p	A	A	A	A
RTs	nd	A	nd	p	nd	p	nd	P	p	nd	nd	nd	nd
ST	nd	nd	nd	p	nd	p	nd	P	p	nd	nd	nd	nd
LVs	p	nd	nd	p	p	p	nd	p	p	nd	nd	nd	nd

AKs = alkaloids, TPn = terpenes, TPd = terpenoids, TNs = tannins, SPn = saponins, GSd = glycosides, ANq = anthraquinones, PHn = total phenolics, FNd = flavonoids, PBt = phlobatannins, ANc = anthocyanins, CRn = coumarins, STd = steroids, WP = whole plant, RTs = roots, STs = stem, LVs = leaves, p = present, A = absent and nd = not done

Table 6. Concentration of phytochemicals in whole plant and parts of *E. granulata.*

Phytochemicals	Quantitative composition (mg/g) of plant and parts of extract			
	Whole plant	Roots	Stem	Leaves
Total Phenolics (GAE)	0.8 ± 0.025	0.1 ± 0.012	0.3 ± 0.015	1.1 ± 0.012
Flavonoids (QE)	0.7 ± 0.02	0.1 ± 0.0	0.4 ± 0.015	0.9 ± 0.0
Alkaloids (PE)	0.7 ± 0.006	0.1 ± 0.0	0.5 ± 0.025	0.8 ± 0.015
Tannin (TAE)	0.2 ± 0.012	0.0 ± 0.0	0.2 ± 0.02	0.6 ± 0.01
Glycosides (DE)	0.6 ± 0.025	0.3 ± 0.173	0.6 ± 0.025	0.7 ± 0.035
Saponins (QSE)	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.025

Table 7. Concentration of phytochemicals in whole plant and plant parts of *E. prostrata.*

Phytochemicals	Quantitative composition (mg/g) of plant and parts of extract			
	Whole plant	Roots	Stem	Leaves
Total Phenolics (GAE)	0.1 ± 0.153	0.2 ± 0.015	0.7 ± 0.035	1.2 ± 0.025
Flavonoids (QE)	0.5 ± 0.03	0.0 ± 0.0	0.3 ± 0.015	0.7 ± 0.025
Alkaloids (PE)	0.6 ± 0.015	0.0 ± 0.0	0.3 ± 0.0	0.9 ± 0.025
Tannin (TAE)	0.3 ± 0.015	0.1 ± 0.006	0.2 ± 0.0	0.7 ± 0.025
Glycosides (DE)	0.9 ± 0.025	0.2 ± 0.01	0.4 ± 0.025	1.2 ± 0.006
Saponins (QSE)	0.2 ± 0.006	0.0 ± 0.0	0.0 ± 0.02	0.3 ± 0.006

Values are mean of three with ± SD, GAE = gallic acid equivalent, QE = quercetin equivalent, PE = papaverine equivalent, TAE = tannic acid equivalent, DE = digitoxin equivalent and QSE = quillaja saponin equivalent

Table 8. Mineral composition of representative soil sample and permissible limits in soil.

Mineral	Concentration in soil	Permissible concentration for soil	Reference source
Fe	23233 ± 504.4	129000ppm	Ramamurthy & Kannan (2009)
Mn	516 ± 12.01	1000ppm	Ramamurthy & Kannan (2009)
Zn	226 ± 8.8	300ppm	Ramamurthy & Kannan (2009)
Cu	2.7 ± 0.06	100ppm	Ramamurthy & Kannan (2009)
Mo	1.5 ± 0.019	4ppm	Chesworth, (2008)
Ca	33200 ± 86.8	52000ppm	Ramamurthy & Kannan (2009)
P	27116 ± 116.66	Not specified	-
Mg	5433 ± 60.09	9000ppm	Ramamurthy & Kannan (2009)
S	45633 ± 88.19	Not specified	-
N	Not done	Not specified	-
K	23150 ± 86.60	37000ppm	Ramamurthy & Kannan (2009)
Na	19050 ± 86.60	25000ppm	Ramamurthy & Kannan (2009)
Cl	1596 ± 8.8	Not specified	-
Se	1.4 ± 0.05	2ppm	Ramamurthy & Kannan (2009)

Values are the mean of 3 replicates with ± SEM

Table 9. Distribution of minerals elements in whole plant and plant parts of *E. granulata.*

Mineral	Mineral composition in ppm				
	Whole plant	Roots	Stem	Leaves	Seeds
Fe	803.3 ± 4.4	416 ± 2.96	682 ± 2.33	919 ± 2.08	105 ± 0.75
Mn	65.1 ± 0.38	59.2 ± 0.11	64.43 ± 0.23	68.9 ± 0.8	18.53 ± 0.088
Zn	47.1 ± 0.09	52.7 ± 0.12	45.0 ± 0.07	40 .0 ± 0.12	19.45 ± 0.058
Cu	BDL	1.9 ± 0.02	BDL	0.9 ± 0.02	0.66 ± 0.017
Mo	1.9 ± 0.03	2.3 ± 0.03	1.5 ± 0.29	2.1 ± 0.04	BDL
Ca	3007 ± 14.5	2167 ± 12.01	3223 ± 20.27	4130 ± 15.27	3164 ± 7.44
P	1638 ± 6.0	1378 ± 2.08	1605 ± 7.63	1998 ± 6.0	1942 ± 4.41
Mg	4883 ± 20.27	2273 ± 6.66	4970 ± 5.77	5265 ± 7.63	2198 ± 6.0
S	31183 ± 60.09	36157 ± 34.8	26500 ± 76.37	35633 ± 60.09	21070 ± 17.32
N	2.80 ± 0.02	2.75 ± 0.009	2.52 ± 0.006	3.15 ± 0.01	3.58 ± 0.012
K	12533 ± 109.3	2571 ± 10.9	12800 ± 15.275	9675 ± 7.638	1332 ± 4.41
Na	3498 ± 19.22	4261 ± 4.41	2885 ± 7.63	882 ± 7.26	470 ± 5.23
Cl	BDL	1.98 ± 0.06	BDL	BDL	BDL
Se	34.46 ± 0.08	89.8 ± 0.04	23.57 ± 0.06	27.81 ± 0.038	2.78 ± 0.043

Table 10. Distribution of minerals elements in whole plant and plant parts of *E. prostrata*.

Mineral	Mineral composition in ppm				
	Whole plant	Roots	Stem	Leaves	Seeds
Fe	1112.6 ± 9.93	578 ± 9.28	1052 ± 10.13	1395 ± 7.6	165.5 ± 0.76
Mn	46.4 ± 0.03	41.0 ± 0.86	45.6 ± 0.49	51.3 ± 0.43	14.6 ± 0.13
Zn	72.5 ± 0.73	85.8 ± 0.6	68.6 ± 0.30	60.4 ± 0.53	19.4 ± 0.05
Cu	BDL	1.33 ± 0.03	BDL	1.13 ± 0.06	3.3 ± 0.06
Mo	2.0 ± 0.05	2 ± 0.04	1.49 ± 0.2	2.3 ± 0.019	BDL
Ca	3017 ± 20.27	2516 ± 31.8	3453 ± 14.53	4480 ± 51.31	3381 ± 8.02
P	1844 ± 21.85	1310 ± 11.5	1737 ± 23.33	2203 ± 8.81	1855 ± 7.6
Mg	4400 ± 132.29	3350 ± 28.8	4280 ± 20.0	5100 ± 57.73	3255 ± 5.7
S	33550 ± 275.4	37500 ± 288	24933 ± 233.3	33100 ± 378.6	2346 ± 4.9
N	2.92 ± 0.031	2.79 ± 0.06	2.62 ± 0.007	3.39 ± 0.007	3.77 ± 0.017
K	9100 ± 50	465 ± 8.66	12400 ± 208.1	6983 ± 44.1	1367 ± 3.3
Na	1110 ± 22.68	2610 ± 23.1	1377 ± 14.5	351.6 ± 7.26	249 ± 2.02
Cl	BDL	1.7 ± 0.1	BDL	BDL	BDL
Se	74.7 ± 1.12	144 ± 0.66	60.6 ± 1.2	68.66 ± 0.66	27.1 ± 0.11

Values are the mean of 3 replicates with ± SEM

Table 11. Recommended Dietary allowances (RDA) and permissible limits (PL) for minerals.

Mineral	RDA (mg/day)	Reference and authority	PL in edible plants and authority	Reference source
Fe	8.0	Gropper *et al*. (2009); NAS, (2005)	20ppm (FAO / WHO)	Mtunzi *et al*. (2012)
Mn	2.3	Gropper *et al*. (2009); NAS, (2005)	2ppm (FAO / WHO)	Mtunzi *et al*. (2012)
Zn	11.0	Gropper *et al*. (2009); NAS, (2005)	27.4ppm (FAO / WHO)	Jabeen *et al*. (2010)
Cu	0.9	Gropper *et al*. (2009); NAS, (2005)	3ppm (FAO / WHO)	Mtunzi *et al*. (2012)
Mo	0.045	Gropper *et al*. (2009); NAS, (2005)	0.02ppm (FAO / WHO)	Jabeen *et al*. (2010)
Ca	1000	Gropper *et al*. (2009); NAS, (2005)	Not specified	Duruibe, (2010)
P	700	Gropper *et al*. (2009); NAS, (2005)	Not specified	-
Mg	400-420	Gropper *et al*. (2009); NAS, (2005)	Not specified	-
S	Not specified	Dietary mineral	Not specified	-
N	56000 (proteins)	Gropper *et al*. (2009); NAS, (2005)	Not specified	-
K	4700	Gropper *et al*. (2009); NAS, (2005)	Not specified	-
Na	1300-1500	Gropper *et al*. (2009); NAS, (2005)	Not specified	-
Cl	2300	Gropper *et al*. (2009); NAS, (2005)	Not specified	-
Se	0.055	Gropper *et al*. (2009); NAS, (2005)	Not specified	-

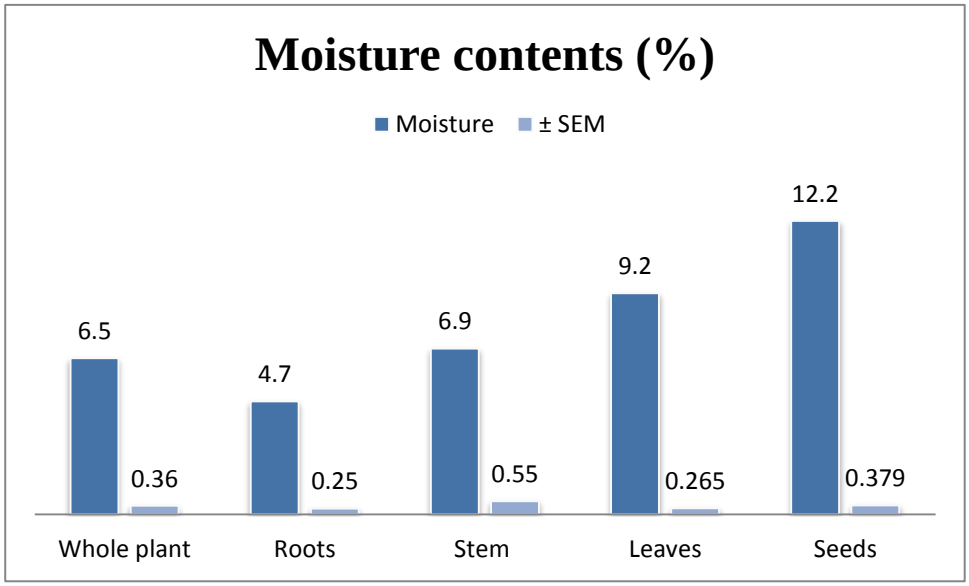

Fig. 4. Moistures content (± SEM) in *Euphorbia granulata* plant and parts.

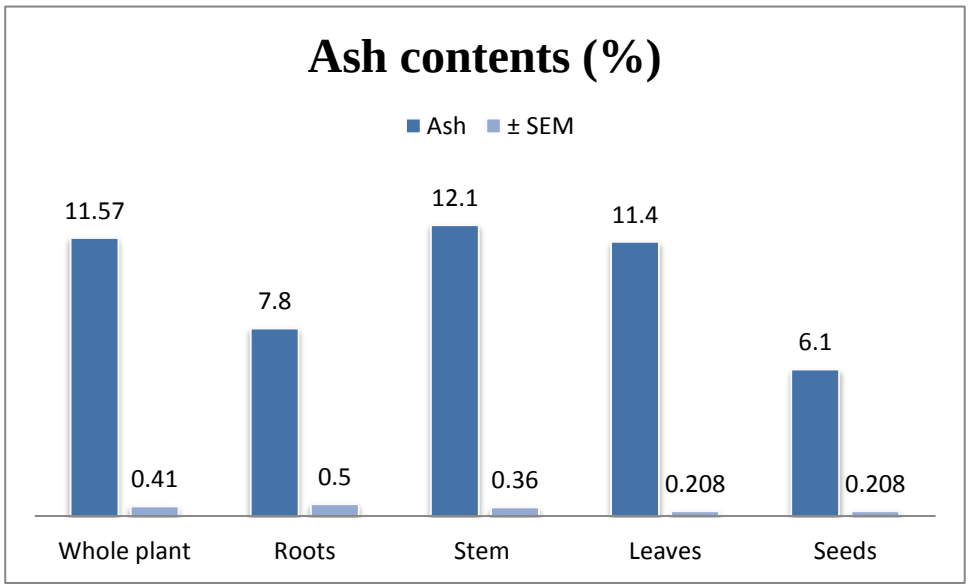

Fig. 5. Ash contents (± SEM) in *Euphorbia granulata* plant and parts.

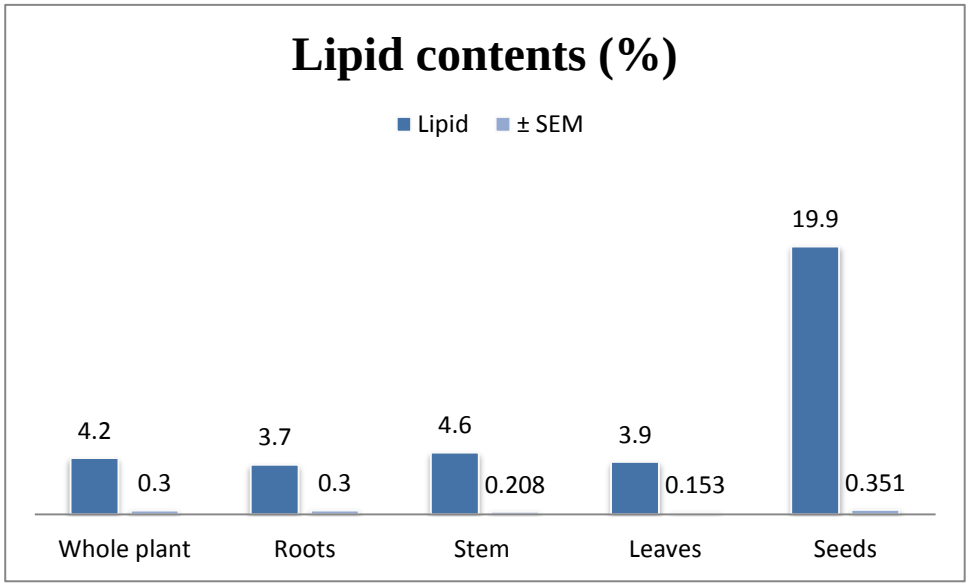

Fig. 6. Lipid contents (± SEM) in *Euphorbia granulata* plant and parts.

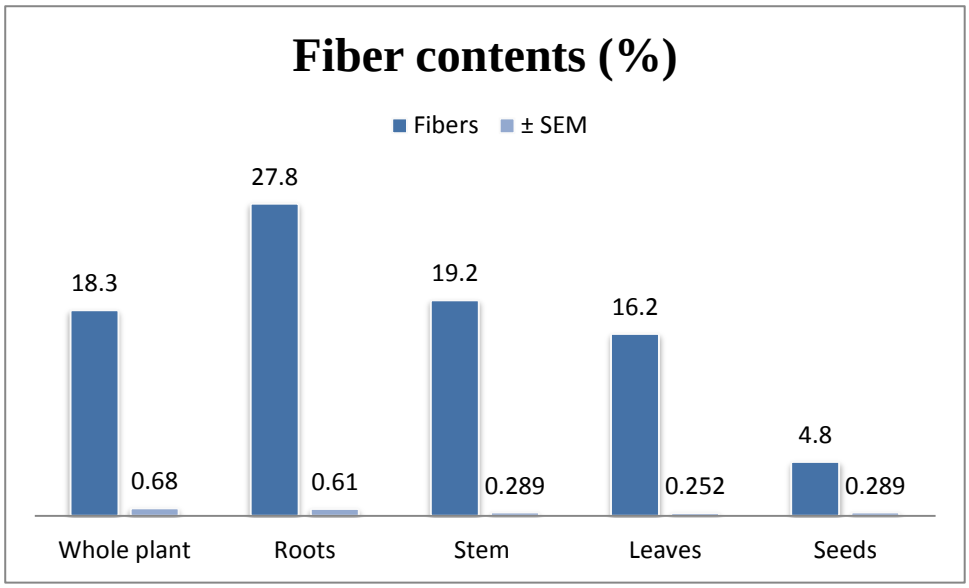

Fig. 7. Fiber contents (± SEM) in *Euphorbia granulata* plant and parts.

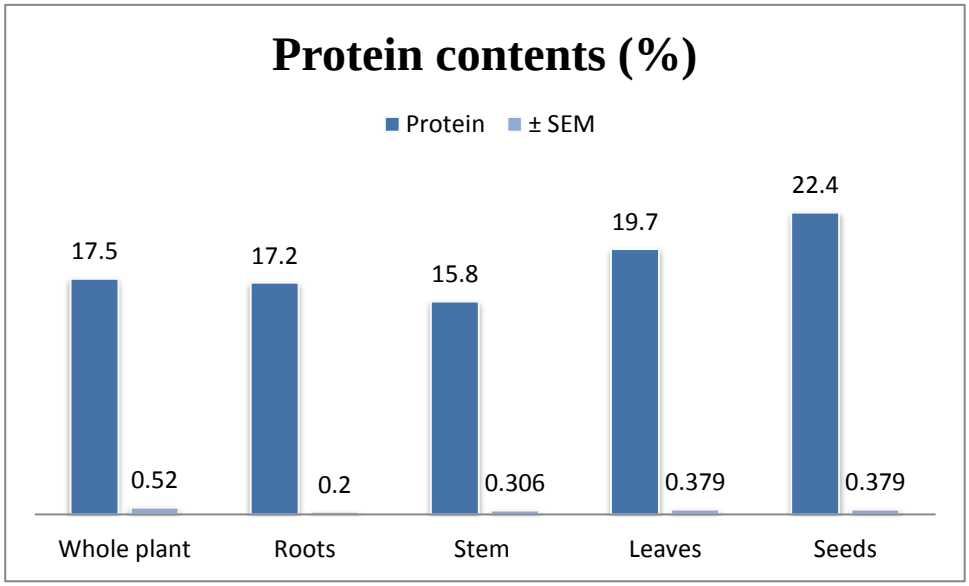

Fig. 8. Protein contents (± SEM) in *Euphorbia granulata* plant and parts.

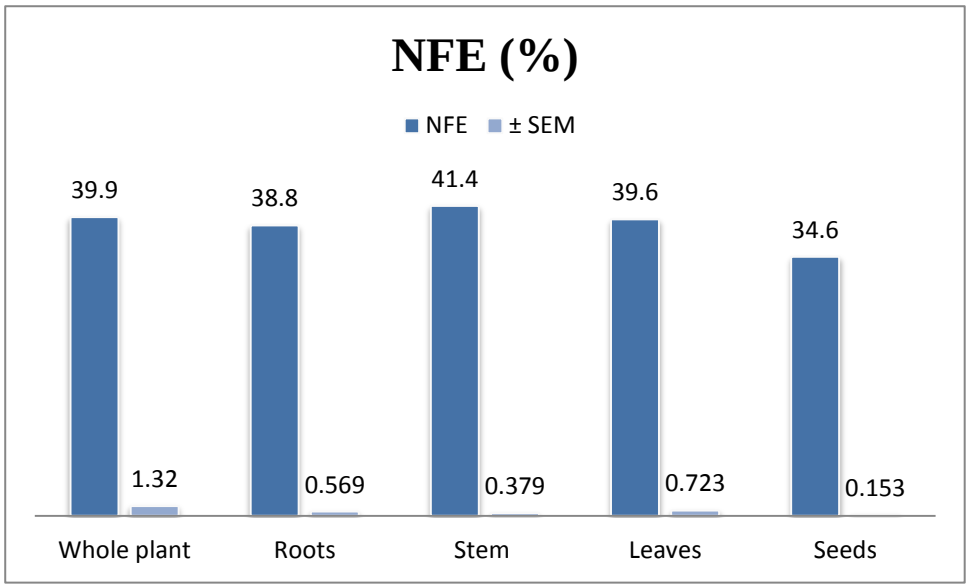

Fig. 9. Carbohydrate contents (± SEM) in *Euphorbia granulata* plant and parts.

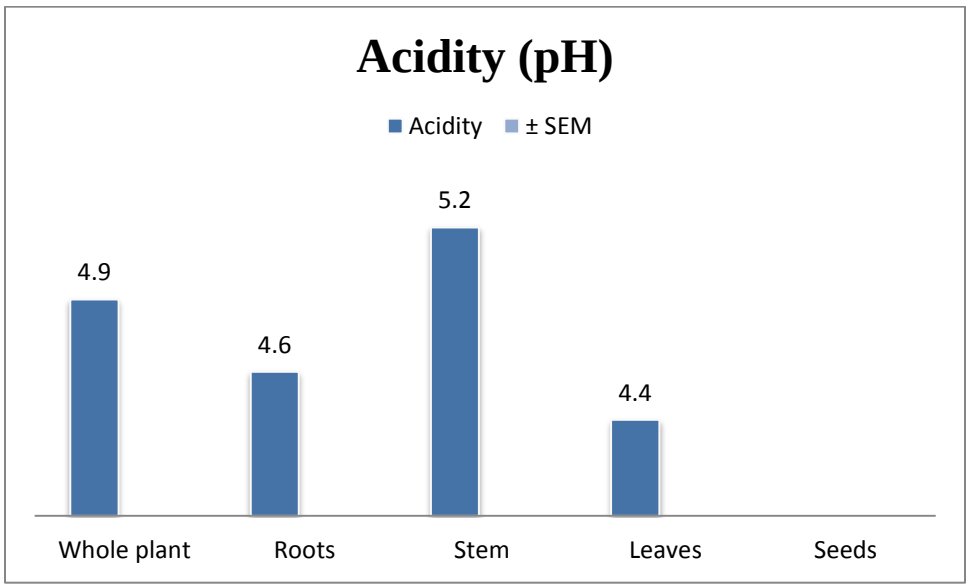

Fig. 10. Acidity (pH) of *Euphorbia granulata* plant and parts.

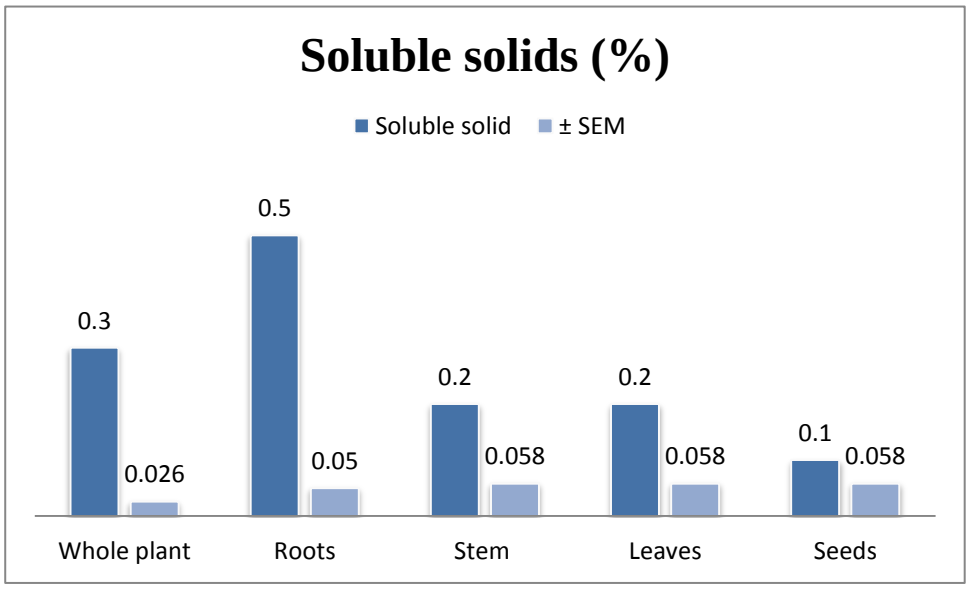

Fig. 11. Soluble solids (%) (± SEM) in *Euphorbia granulata* plant and parts.

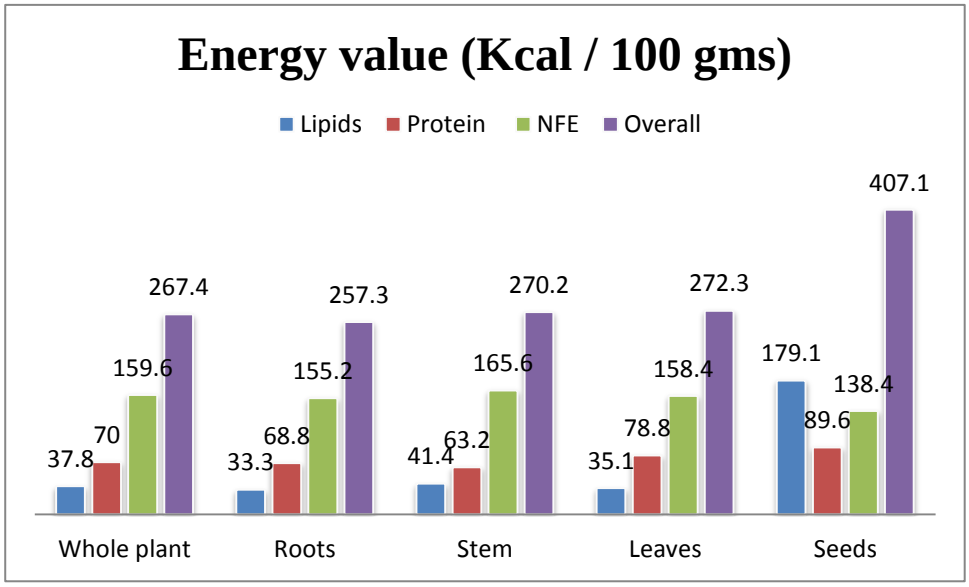

Fig. 12. Energy value of *Euphorbia granulata* whole plant and parts.

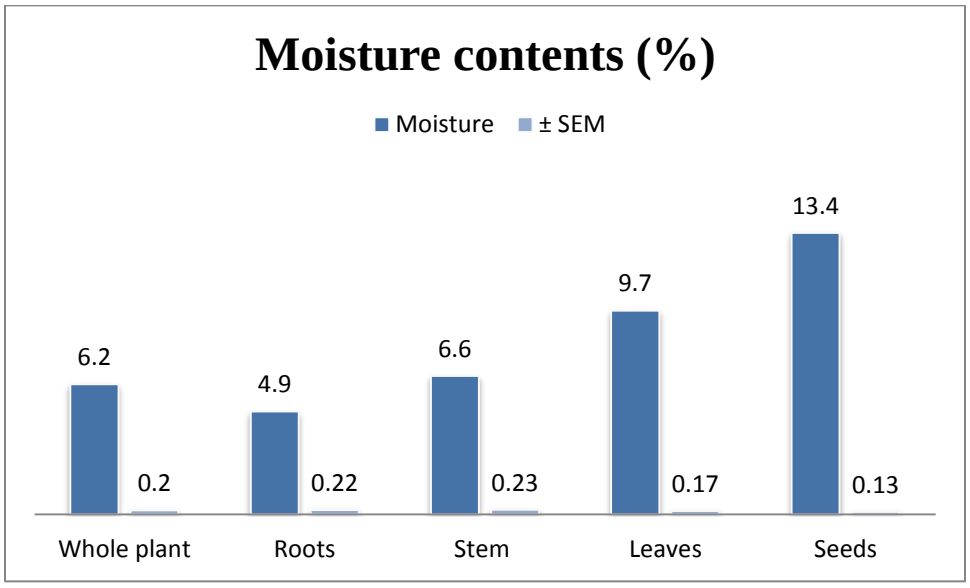

Fig. 13. Percent moisture in *Euphorbia prostrata* whole plant and parts.

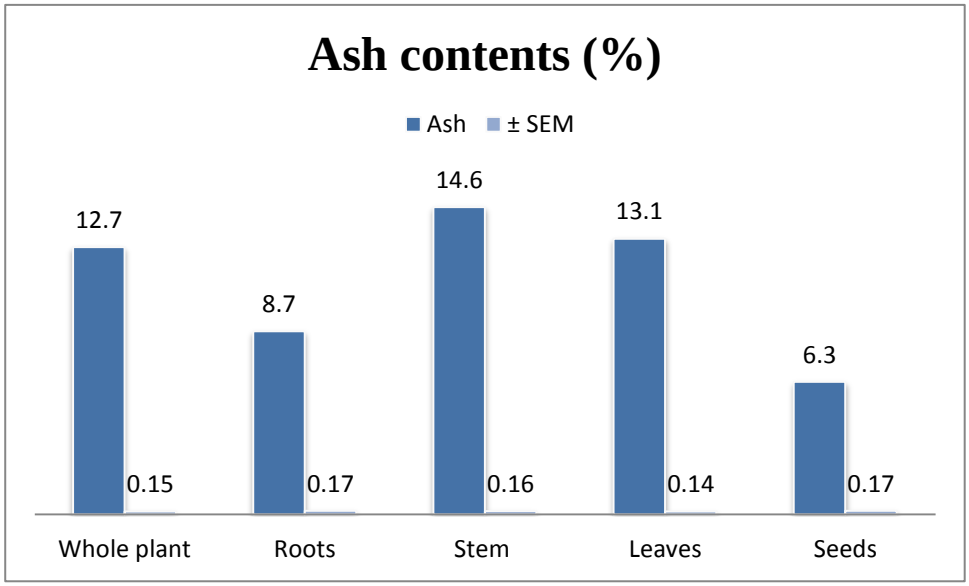

Fig. 14. Percent ash in *Euphorbia prostrata* whole plant and parts.

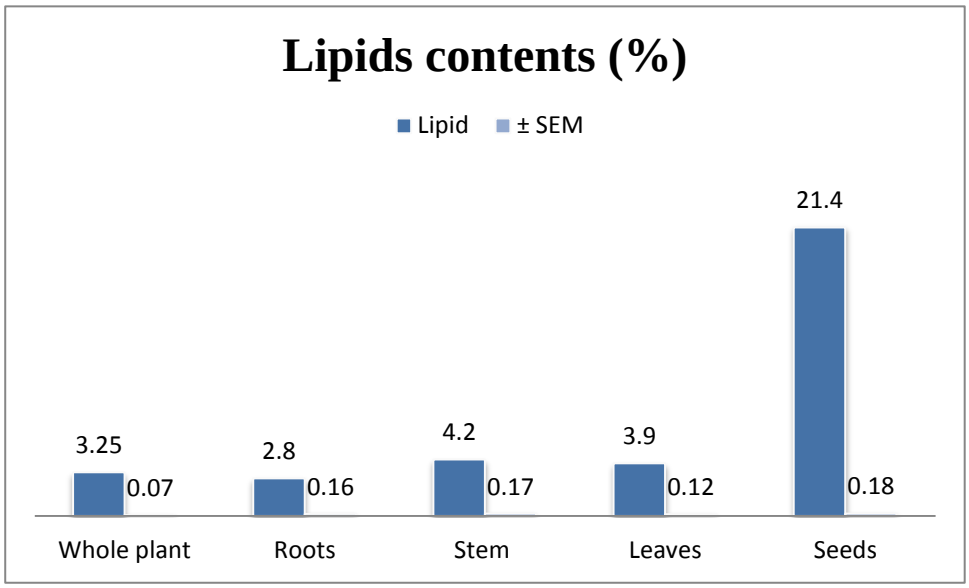

Fig. 15. Percent lipids in *Euphorbia prostrata* whole plant and parts.

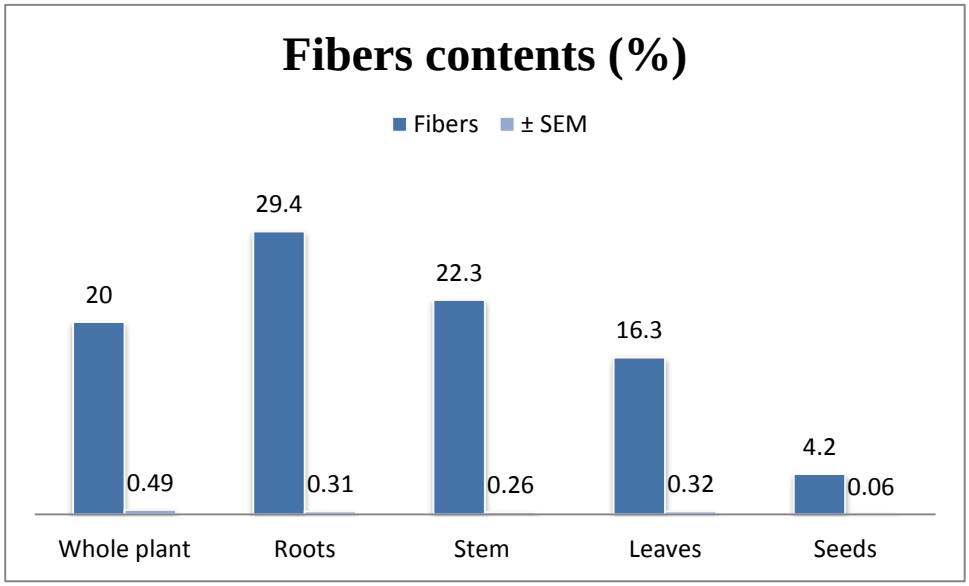

Fig. 16. Percent fibers in *Euphorbia prostrata* whole plant and parts.

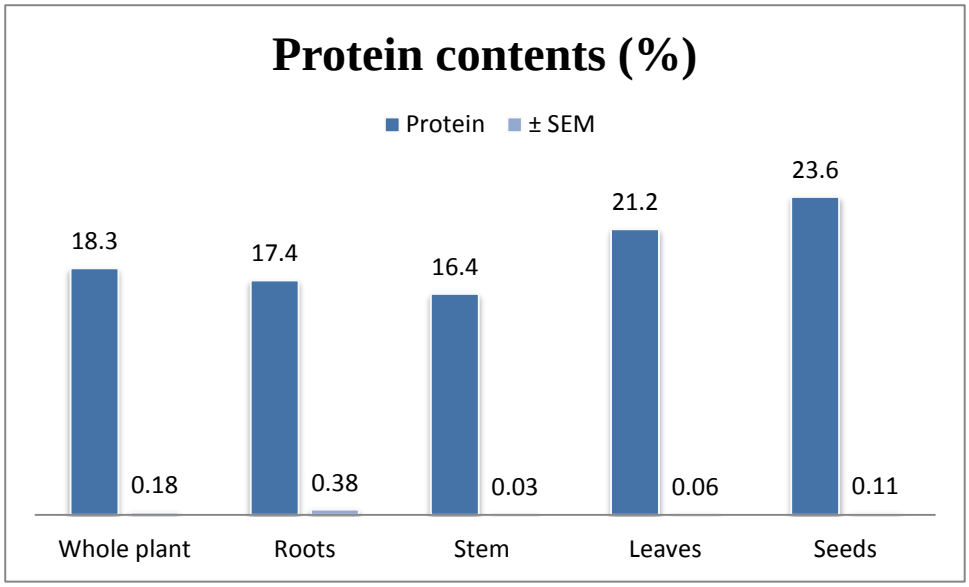

Fig. 17. Percent fibers in *Euphorbia prostrata* whole plant and parts.

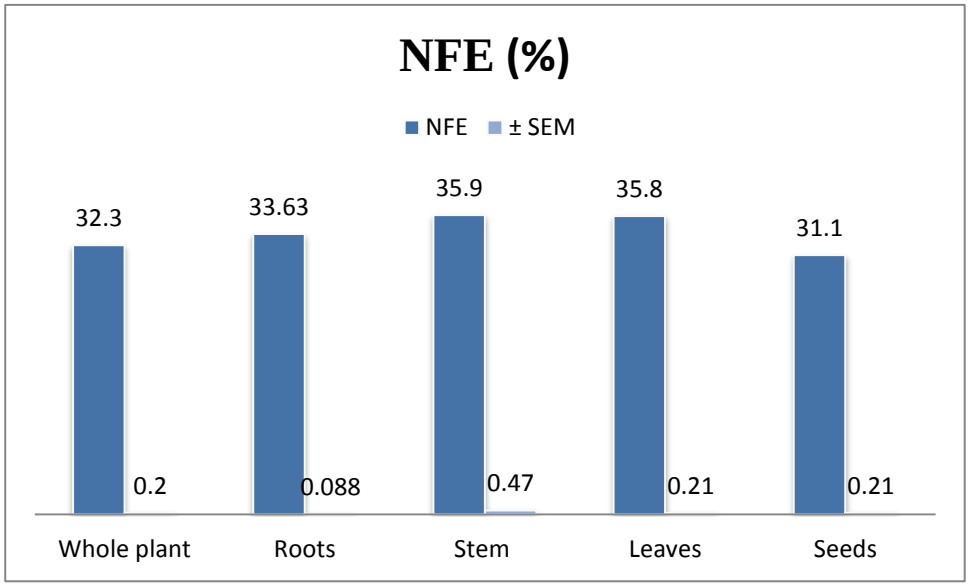

Fig. 18. Percent NFE in *Euphorbia prostrata* whole plant and parts.

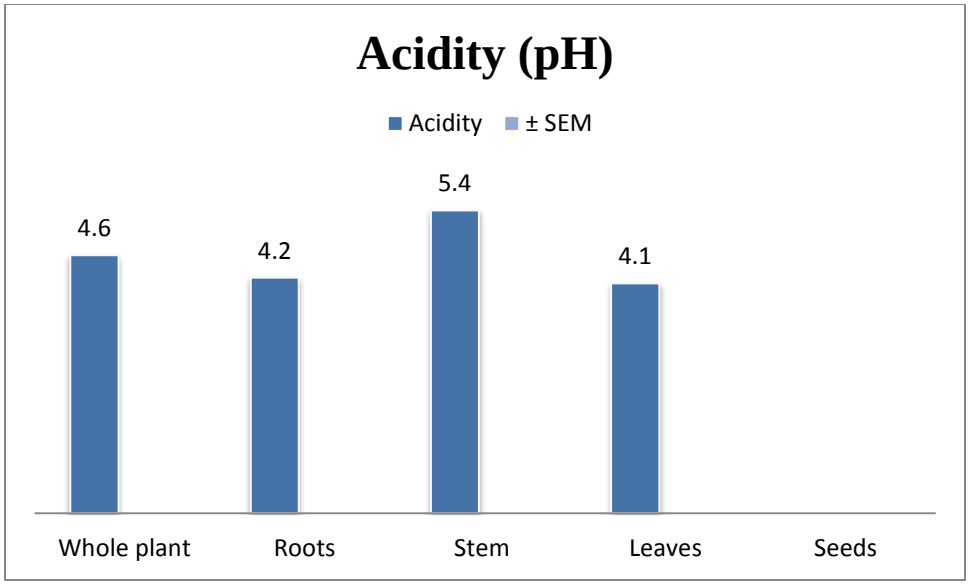

Fig. 19. Acidity in pH for *Euphorbia prostrata* whole plant and parts.

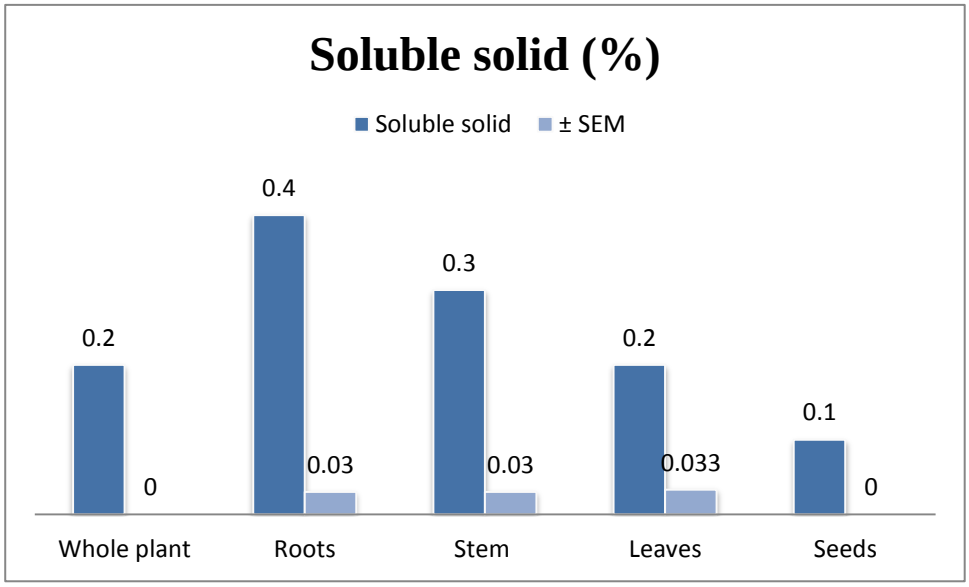

Fig. 20. Percent NFE in *Euphorbia prostrata* whole plant and parts.

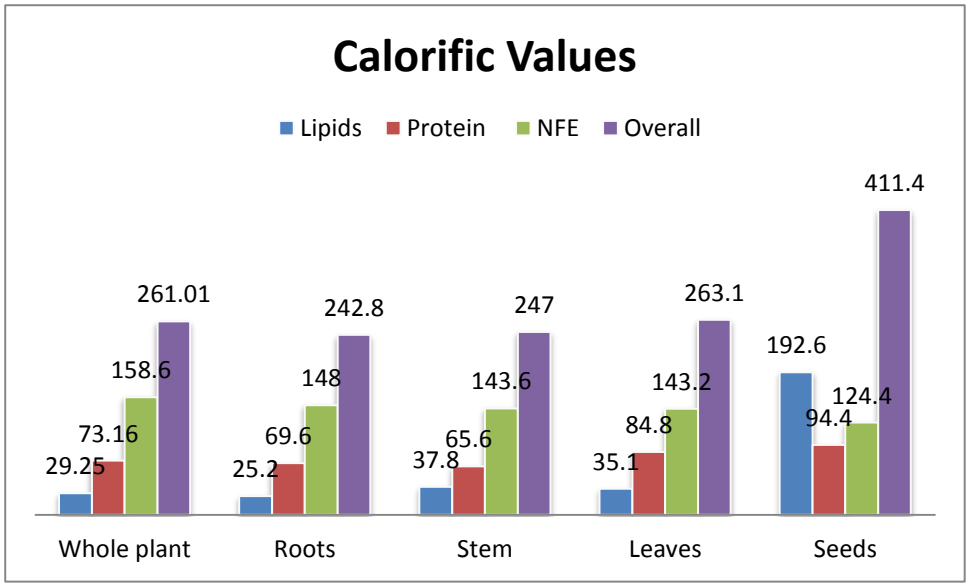

Fig. 21. Calculated energy value of *Euphorbia prostrata* whole plant and parts.

4. Toxic Heavy Metals and Phyto-extraction

Euphorbia granulata Forssk

To see the contamination level of *E. granulata* with toxic heavy metals and to check the plant suitability for extracting these metals from contaminated soil, the plant and representative soil samples were analyzed. The levels of different heavy metals found in representative soil samples are shown in Table 12. The amount of these metals absorbed by the plants and its distribution in plant parts is presented in Table 13. Based on the level of a metal in the soil and in plant, bioaccumulation co efficient (BAC) was calculated for each metal. The values for each parts and each metal are summarized in Table 14. The daily permissible limits with recommended authority are provided in Table 15. The quantity of the metals that could be ingested with plants, collected from different soil samples are presented in Table 16.

Heavy metal composition of Soil

Soil pH, organic matter, chemistry, humus, physical shape, size and water source affect the availability of heavy metal in the soil (Yap *et al*., 2009; Knezevic *et al*., 2009). Table 12 shows the concentration of heavy metals in representative soil samples for each plant collected different areas. The concentration of ten toxic heavy metal in soil for sample 1 was in the order of; Hg > As > Pb > Cd > Co > Cr = Ag > Mo > Ni > Sn; for sample 2 was Hg > As > Pb > Cd > Ag > Co > Mo > Cr > Ni > Sn and for sample 3 it was Hg > As > Cd > Pb > Cr > Co = Ag > Mo > Ni > Sn. Comparing individual metals ratio in different soil samples, Pb, Cd and Hg was noted in high quantities (5.20, 3.60, 21.5ppm, respectively) in sample 1; Ag and Mo (2.3 ppm, 2.0 ppm) in sample 2; As and Cr was high (2.20ppm, 50.50ppm) in sample 3; while Co and Ni were high in soil samples 1 and 2 (2.20ppm, 0.12ppm) i.e. present in equal concentrations in both samples. Tin was found below detectable limit in all the three soil samples. The heavy metal composition of plant collected from footpath (soil sample 1) is mostly influenced by the smoke emitted from the vehicle from the nearby road (Hussain *et al*., 2010) and by organic volatile metals from air. Shoes stuck materials of people walk through may also influence composition. As they are dependent on occasional rain, hence no leaching with water, therefore they contain majority of the metal (Pb, Hg, Cd, Co, Ni) in high quantity.

The streamside (soil sample 2) and garden soil (soil sample 3) is under the influence of frequently running water that leaches the metal. Therefore out of the ten metals only three metals (Ag, Co, Ni) were in high concentration in soil sample 2 and two metals (As, Cr) in soil sample 3. As roots exudates makes a complex with heavy metals and prevent them from leaching, it might have influenced the high presence of three heavy metals in sample 2.

Heavy metal composition of plant

Solvents affect availability of metals and various plant species react differently to different heavy metals in soil (Szabo *et al*., 2008; Hajiboland, 2005; Knezevic *et al*., 2009). Same plant species collected from different soil type's shows different affinities for the same metal; and similarly different parts of the same plant differs for metal accumulation (Kumar *et al*., 2008; Knezevic *et al*., 2009). Therefore, same plant from different soil sample and same metal and its parts were analyzed. The plants with high BAC value indicate their capability to clean soil or water (Hajiboland, 2005) by absorption. A plant may have high BAC value for a particular metal but may not be a good cleaner. This happens when a metal exist in small amount in soil and the plant need it in large amount, therefore the plant actively collect the required metal from soil. Sometimes a plant may have a metal in high concentration and theoretically it may cause toxicity but actually the toxicity relates to the ingested quantity. Therefore, the ingested quantity of a metal with plant consumption should be calculated. For a medicinal plant this could be achieved by calculating the possible daily intake (PDI) of the plant with therapeutic dose of the plant. Heavy metal composition and its distribution in different parts are discussed below.

Lead: Comparatively high quantity (3.5 ± 0.033) of lead was recorded in plant collected from streamside (Table 13). The soil sample had 3.3 ± 0.015 ppm of lead (Table 12). This gives BAC value of 1.06 (Table 14). BAC values noted for footpath and garden were 0.52 and 0.91 respectively. Generally, the BAC value of 1 represents equal concentrations of metal in plant and soil. The value higher than 1 indicate that the plant is efficient absorber of metal and have high concentration against the concentration in soil. Any value below 1 shows poor extraction of metal and hence poor role in soil cleaning.

The present study suggests poor role of *E. granulata* in removing Pb from soil. Comparing lead accumulated in different parts of *E. granulata* showed high concentrations in roots (4.1 ± 0.083 ppm). This trend was noted for all the three plant samples. Plants with more lead when consumed can results toxic accumulation. On investigation from locals it was found that *E. granulata* is consumed from 4 to 5 mg daily for 7 to 10 days in various ailments. By calculating the Pb amount with ingested plant it was found that 23.2 (10-6) to 38.6 (10-6) mg is the possible daily intake with plant (Table 16). The permissible limit for Pb ingestion with edible food is up to 0.025 mg daily (FAO/WHO, 2005). This comparison suggests that the lead amount in plant is less than toxicity levels.

Cadmium: Cd was detected only in roots of *E. granulata*. It was further noted that all the three root samples had almost equal quantities (3.6 ± 0.015). Plant roots produces exudates in rhizosphere that have the ability to makes chelates with metals (Aziz *et al*., 2011). The present accumulation of Pb in plant roots might be the result of such a complex with Cd. The lower tendency of plant for Cd shows that *E. granulata* was not a good bio-accumulator. The daily maximum permissible limit (FAO/WHO, 2005) for ingestion is up to 0.007 mg of Cd. Although the BAC (1.44) value for roots is higher (Table 14), but the ratio of roots to the whole plant is too small. Therefore it will not affect the overall concentration of plant. The present results indicate that ingestion of Cd with plant may not be risky to its consumer.

Chromium: Chromium is heavy metal which is also an important micro-mineral at concentration within permissible limit. However it becomes toxic when taken in large amount. Daily ingestion of 0.25 mg (Table 15) is considered as safe, but quantity beyond this limit may cause toxic effects (WHO, 1996). The level of chromium in *E. granulata* was not enough to be detected (Table 13). Small amounts of 0.6 ± 0.015 (footpath sample) and 0.2 ± 0.012 mg (streamside & garden samples) were noted in roots only. The BAC value calculated for root was far below 1. Therefore *E. granulata* is a poor chromium phyto-extractor. The amount present in roots is enough to contribute towards the daily need of its consumer without any hazardous affects with therapeutic dose.

Cobalt: It is essential part of vitamin B12 and has no other known role in the body. High levels in the body might be harmful (Khan *et al*., 2011; Hussain *et al*., 2010). US National Research Council (US-NRC) have set 2 mg/kg as the maximum limit for animals. The average daily intake of Americans is 0.005-0.04 mg; whereas European Food Safety Authority (EFSA, 2012) has not specified permissible limit for human. The results revealed *E. granulata* has Co levels between 0.10 ± 0.003 and 0.12 ± 0.003 ppm. Roots had the maximum concentration. With this much quantity in whole plant (0.10 ± 0.003 to 0.12 ± 0.003 ppm) the calculated maximum PDI is 1.32 (10-6) ppm (Table 16). The data suggest that the present level in the plant is well within the permissible limits. Further consideration of Co levels in soil (2.3 ± 0.009) and plant body (0.12 ± 0.003) revealed that very small fraction of Co is absorbed by plant from soil. The maximum BAC value was 0.05 which suggests a very poor role of plant in phyto-extraction.

Silver: The maximum levels of silver recorded for *E. granulata* was 1.8 ± 0.015. Of this total silver in whole plant, the roots had the maximum concentration (2.1 ± 0.05). Stem contained the least (1.0 ± 0.022). BAC value calculated against the concentration in soil (2.0 ± 0.060 ppm) was 0.9, which suggest lower affinity of plant for silver. Furthermore, at this Ag concentration in whole plant, the calculated ingestion would hardly be 19.87 (10^{-6}) mg per day (Table 16). The safe daily permissible limit is up to 0.39 mg (EFSA, 2005) which suggests that the plant might be consumed without Ag associated hazards.

Mercury: Hg had the highest concentration both in soil (21.5 ± 0.017 ppm) and in *E. granulata* whole plant (29.36 ± 0.056 ppm). The highest BAC (2.2) was recorded for garden sample following by 1.37 by footpath sample. The distribution of mercury was in pattern of; roots > whole plant > stem > leaves for footpath sample, whole plant = roots > stem > leaves for streamside sample and whole plant = leaves > stem > roots >for garden sample. The results suggest that *Euphorbia granulata* has the potential for extracting Hg from soil. The data further revealed that high metal concentration was found in plants growing in soil with high mercury contents. This suggests a concentration dependent absorbance. But it was observed that in all samples more Hg accumulates in plant body than present in the soil. The safety of the plant in terms of Hg concentration is

also important. Data in Table 16 revealed that the plant with therapeutic dose will hardly deliver 220 (10-6) to 324.1 (10^{-6}) mg daily. This is a safe ingestion quantity against the permissible limits (Up to 0.005 mg) as suggested by FAO/WHO (2005).

Arsenic: After mercury, arsenic was the most abundant element in soil, recorded in the present study. The concentration in soil ranged from 7.25 ± 0.007 in footpath sample to 7.25 ± 0.007 ppm in streamside sample. Proportionally good amount was also found in *E. granulata* whole plant and plant parts. In whole plant As quantity ranged from 2.25 ± 0.023 to 2.92 ± 0.012 ppm. The present concentration in whole plant resulted in BAC value of 0.46 which indicates poor phytoextraction for arsenic. Roots accumulated high levels (9.43 ± 0.009 ppm) with BAC value of 1.3. Once arsenic was thought as poisonous metal but recent evidences classify this metal into essential trace elements. The reasonable quantity of arsenic in *E. granulata* can be of benefit for methionine metabolism (Gropper *et al*., 2009). The permissible daily quantity of arsenic in food is up to 0.15 mg (FAO/WHO, 2005). However, high amount may produce toxicity and even death (Duruibe *et al*., 2007). The present concentration in plant is enough to deliver 24.8 (10-6) to 32.2 (10-6) ppm of As (Table 16). This concentration is within the permissible range that proves the plant for safe consumption.

Molybdenum: The quantity of Mo in whole plant and parts along with its importance has already been discussed with mineral nutrients (section C). It was calculated that only 20.98 (10^{-6}) mg daily will be delivers to a 60 kg body person with therapeutic dose (Table 16). The daily permissible limit, recommended by National Research Council-USA is 0.075-0.25 mg (FNB, 1989). The concentration in *E. ganulata* suggests that it will not produce toxicity.

Footpath sample had more Mo in plant body (1.9 ± 0.015 ppm) than in soil (1.5 ± 0.019). These values results in 1.27 BAC value. This suggests that the plant extract more molybdenum than its concentration in the soil. The results suggest that *E. granulata* might have capability of cleaning Mo from contaminated soil.

Nickel: It is an essential mineral nutrient but concentration above permissible level is toxic. The unwanted effects associated with nickel toxicity include allergic dermatitis or nickel itch (Hussain *et al*., 2011; Hussain *et al*., 2010; Khan *et al*., 2008).

All the three samples were observed with 0.13 ± 0.003 ppm of Ni. Distribution in plant parts was found as; roots > w. plant = leaves > stem (Table 13). Similar pattern of Ni presence was noted for the entire three samples. This amount was almost equal to the amount fount in soil samples. The high nickel contents of the roots were also reported in maize by Szabo *et al*, (2008). This suggests that there might be some natural retaining ability in roots for Ni. Recommended daily safe consumption for Ni is 0.15 mg (EFSA, 2006). The calculated possible daily intake with plant is 1.4 (10^{-6}) mg /day (Table 16). The present data shows that *E. granulata* could be safe in terms of Ni toxicity. The amount of Ni present in representative soil sample was too small but similar quantity in plant reveals the affinity of plant for Ni. The BAC value calculated for nickel was above 1 for entire three samples. This further strengthened the belief that the plant has the tendency to take up Ni from soil even if it is present at low concentration.

Tin: Tin was not detected in none of soil samples tested. It also was neither present in whole plant nor in its parts. Up to 14 mg/day/person is the permissible limit for tin (FAO/WHO, 2005). Tin is not needed in the body.

***Euphorbia prostrata* Aiton**

World Health Organization recommends that medicinal plants be checked for different contaminants including metals before using therapeutically (Mtunzi *et al*., 2012). The present result shows that the investigated plant takes up various heavy metals differentially. Tin was below detectable limit (BDL) in soil samples while the rest nine metals were detectable. The concentration of all the HM varied in the soil samples, the whole plant and plant parts (Tables 12, 17). The distribution of each element with calculated BAC value in soil samples, whole plant and plant parts is discussed below.

Heavy metal composition of Soil samples: The levels of heavy metals in representative soil samples have already been discussed with *E. granulata*.

Heavy metal composition of plant samples: The levels and importance of heavy metals in plants and its distribution in different plant parts are discussed with *E. granulata*. The quantities of analyzed metals in plants are discussed here.

Lead: is a highly poisonous heavy metal which accumulates but no known function in the body (Duruibe *et al*., 2007; Hussain *et al*., 2010). Lead poisoning results

in nephritis, brain damage, CNS disorders, anemia and colic (Khan *et al*., 2008). The present results shows heavy metal distribution in *Euphorbia prostrata* as; roots > w. plant > leaves > stem for footpath sample, roots > w. plant = stem > leaves for streamside sample and roots > w. plant > stem > leaves for garden sample. The highest quantity of lead was found in roots (3.45ppm) from soil sample 1 (Table 17). High value in the roots and poor translocation to the aerial parts agrees with the results obtained for water hyacinth by Aisien *et al*, (2010). The high accumulation of heavy metals in roots might be due to the plant exudates that makes metal-chalate complexes in the root zone (Aziz *et al*., 2011). Environmental role of the plant can be judged from BAC value. The BAC value for Pb was 0.6 for whole plant (Table 18) which indicates that the plant is inefficient in removing lead from the soil. Comparing plants in three soil samples, high concentration was noted for plant grown in soil with high lead concentration. This shows that absorbance of lead from the soil is concentration dependent i.e. the more it is present in the soil the more it is absorbed. Translocation to the aerial parts was not active as comparatively high value was noted for roots.

To evaluate safety of the plant in terms of Pb concentration the daily intake is to be calculated and compared with permissible limits. At present concentration in plant the possible daily intake for a patient consuming 4 to 5 grams of this plant per day is 24.3 (10^{-6}) mg/day (Table 19) while the WHO permissible limit is 0.04-0.05 mg/day (Table 15). This shows that concentration of Pb in *E. prostrata* is far below the toxic level and can be considered as safe for consumption.

Cadmium: In the present analysis Cd was not detectable in whole plant from all samples but had accumulation of 3.4ppm in roots (Table 17) only. High value in the roots and poor translocation to the aerial parts agrees with the results obtained for water hyacinth by Aisien *et al*, (2010). On consumption, Cd accumulates but has no known function in the body (Duruibe *et al*., 2007). Consumption beyond permissible limit can damage kidney and liver. Inhaling the metal causes pneumonia by damaging the lungs (Duruibe *et al*., 2007; Khan *et al*., 2008). If only roots of *E. prostrata* with available concentration is consumed at therapeutic dose this will deliver only 3.3 (10^{-6}) µg / day.

The permissible limit is 0.007 mg/day (FAO/WHO, 2005).The data obtained suggests that *E. prostrata* it not toxic at therapeutic dose.

Furthermore, the results show that *E. prostrata* is not a good Cd accumulator. In all samples Cd was concentrated in roots (BAC value 1.13) and was not detected in the whole plant. This reflects that Cd was not translocated to aerial parts in sufficient quantity and hence indicates a poor role in environments.

Chromium: chromium was also below detecting limit in whole plant but in high concentration (2.50ppm) was noted in the roots from footpath sample (Table 17). Plants and other food items with high Cr concentration may cause Cr toxicity (Eboh & Thomas, 2005). Toxicity affects skin, nasal cavity, stomach, kidney, liver and lungs. Ingestion between 7.2 and 8.4 mg/day (WHO, 1996) Cr is good for regulating glucose, fats and protein metabolism. Chromium is also essential for insulin secretion and its deficiency results in increase blood glucose and cholesterol (Hussain *et al*., 2010). In the present study chromium was detected in roots only. The absence of Cr in whole plant ensures that *E. prostrata* can be used safely for therapeutic purpose. Even if taking only roots of the plant for treatment, the patient will ingest 2.76 (10^{-6}) mg of chromium (Table 19). This amount is far below the permissible limit (250 mg/day) proposed by WHO (1996), therefore the plant can be considered as safe in terms of Cr toxicity. Furthermore, Cr was noted below detecting limit in whole plant. This shows that there was no environmental role of the plant for cleaning Cr. Although with high concentration (2.50 ppm) in roots with BAC value of 1.25 (Table 18) but it will not contribute to environmental role of *E. prostrata*.

Cobalt: Within permissible limits (0.15-0.50mg/day) cobalt is essential for human health. It is a part of vitamin B_{12} and have role in the production of red blood cells. At higher doses it is highly toxic (Khan *et al*., 2011; Hussain *et al*., 2010). Results of the plant analysis show that Co is poorly absorbed from soil, suggesting poor phyto-extraction. Table 18 shows BAC value less than 1 for all samples. High concentration was noted for roots while leaves with poor (Table 17). These results suggest more affinity of the roots for metal than of the shoots. Aziz *et al*. (2011) explained the high accumulation of metals in the roots due to formation of metal-chalate complexes in

rhizosphere. Although, permissible limit is not specified for cobalt (EFSA, 2012), but some authors have specified the maximum limit as 0.15-0.5 mg/day (Shar *et al*., 2007). A person using the plant for therapeutic purpose will receive only 1.1 (10^{-6}) mg of cobalt/day (Table 19) which is less than permissible limit. These figures ensure the safe use of *E. prostrata* as a medicinal plant.

The calculated BAC value (0.06) for cobalt predicts negligible absorbance from soil (Table 18). For stem and leaves BAC value is less than 1; this show poor extraction potential of *E. prostrata* for cobalt. Poor cobalt concentration in the roots than in soil agrees with that of Szabo *et al*, (2008) in the maize plant. This supports our finding of poor absorption from soil by the plant.

Silver: The present analysis showed high concentration in the roots followed by whole plant, leaves and stem respectively. Roots contained 2.9 ppm of Ag (Table 17). WHO (2004) suggest not more than 10 grams of silver ingestion in whole life. The maximum allowable concentration in food (with 1kg/day consumption) is 0.05 mg/kg (EFSA, 2005). Beyond the prescribe limit Ag accumulates in skin, nail, mucous membrane, cornea, brain, lungs, spleen, kidney and liver. It produces reactive oxygen species (ROS) that causes inflammation and cells death. Toxic level results in argyria (irreversible skin pigmentation), systemic argyria, hypersensitivity of skin, erythema multiforme, argyrosis (pigmentation in eye), and allergic contact dermatitis (Panyala *et al*., 2008). From present results it can be claim that *Euphorbia prostrata* is safe in terms of Ag because small amount of plant will deliver 16.6 (10^{-6}) mg per day (Table 19). This amount is far less than per day permissible limit (0.39 mg) recommended by EFSA (2005). This suggests that there is no risk of silver toxicity with therapeutic use.

The plant absorbed silver which is mostly deposited in the roots. This again proves that *E prostrata* is not efficient silver cleaner. The high concentration in roots (2.9 ppm) with BAC calculated value of 1.26. For whole plant BAC value is 0.85 (Table 18). The BAC value less than 1 for the whole plant confirm the poor environmental role of *E. prostrata*.

Mercury: Mercury level found in roots, stem and leaves indicates high affinity of roots for this metal. A concentration dependent absorbance was noted when compared Hg

in three soil samples with that absorbed by the respective plant (Tables 12, 17). Although there is no specific role of Hg in the body but if ingested it keeps on accumulating. High level can results in toxic effects like congenital malformation, Gastro Intestinal disorders, acrodynia or pink disease, brain and CNS damage, gingivitis and stomatitis (Duruibe *et al*., 2007). To avoid hazards of Hg, FAO/WHO (2005) has set 0.005 mg/day as the upper limit for all individuals. With therapeutic dose of *E. prostrata* only 157.9 (10^{-6}) mg (Table 19) is ingested. This suggests that at therapeutic dose ingestion *E. prostrata* will not deliver toxic amount of Hg.

The results of uptake by the plant reveal that *E. prostrata* if a good accumulator of Hg. The BAC value was more than 1 for all the three samples. It was 1.17, 1.33 and 1.43 for sample 1, 2 and 3 respectively (Table 18). BAC values for roots were more than that for the aerial parts. BAC values above 1 suggest that *E. prostrata* has good affinity for silver uptake that may be helpful in cleaning mercury from soil. Moreover comparison of three plant sample revealed that plants absorbed more in soil having high concentration of Hg (concentration dependent) but the positive in the result is that more Hg goes to the plant than it is in the soil.

Arsenic: Arsenic is thought to be a toxic metal but now evidences are accumulating for the essential status of this metal. Reports show the role of As in methionine metabolism and production of heat shock protein. In animal the deficiency curtail growth, reduces conception and increase neonatal mortality. In the body this metal exists in inorganic trivalent (As3+) or pentavalent (As5+) form, mostly in skin, hair and nail (Gropper *et al*., 2009). In the tissues Sn is found attached to thiol/sulfhydryl (SH) group of protein. At toxic concentration Arsenic inhibit ATP production by coagulating important protein and making complexes with coenzymes. Arsenic toxicity often produces similar and symptoms confusing with Guillain-Barre syndrome. High level exposure is considered carcinogenic and fetal (Duruibe *et al*., 2007). The present investigations revealed high concentration in roots (about 7.6 ppm), followed by stem (1.88 ppm) and least (0.38 ppm) in the leaves (Table 17; sample 1). Daily ingestion of this metal with plant was 26.5 (10^{-6}) mg (Table 19). This amount is within the range of permissible limit suggested by FAO/WHO (2005), i.e. up to 0.15 mg/day (Table 15).

This indicates therapeutical safety of *E. prostrata*. BAC value calculated for arsenic was 0.45, (Table 18) for footpath sample. The values less than 1 indicate poor metal extraction. Therefore *E. prostrata* does not seem a good arsenic scavenger.

Molybdenum: Mo is essential for enzymes like xanthine oxidase, nitrate reductase and sulfite oxidase (essential enzymes for electron transport). Within permissible limits Mo help in copper utilization but high intake produces copper deficiency which leads to microcytic anemia (Gropper *et al*., 2009). All metal discussed above were more accumulated in the roots but molybdenum was more accumulated in leaves. Comparing concentration amongst three plant samples, high concentration of (2.0 ppm) was noted in whole plant for footpath sample. Here 2.3 ppm Mo was recorded for the leaves (Table 17). Not only aerial part of the plant was noted for high Mo concentration but BAC value was also above 1 for whole plant and leaves. BAC was 1.33 for whole plant while it was 1.53 for highly concentrated leaves. This trend of high Mo concentration in leaves was also recorded for other two plants samples as well. These results suggest that *E. prostrata* have the capability of cleaning Mo from contaminated soil. High concentration with BAC value more than 1 may not be necessarily toxic to the consumer. Apparently looks to be toxic but when ingested quantity was calculated and compared with permissible limit there was no risk to the consumer health. The calculated possible ingested amount was 17.7 (10^{-6}) mg for a 60kg body (Table 19) while recommended safe use is up to 0.6 mg/day (FNB, 1989).

Nickel: ESFA (2006) suggest 150 mg/day as the maximum allowable quantity for Ni ingestion (Table 15). Data for Ni in *E. prostrata* reveals that Ni had identical status to Cd and Cr i.e. it was recorded in roots only. For roots of footpath sample, 0.13 ppm Ni was recorded. The high nickel contents of the roots were also reported in maize by Szabo *et al*, (2008), which strengthened our present findings. Both high and low ingestion are harmful because Ni is required in minute amount for the production of insulin and proper functioning of liver. Ingestion above permissible limit can produces unwanted effects like allergic dermatitis or nickel itch (Hussain *et al*., 2011; Hussain *et al*., 2010; Khan *et al*., 2008). The level of Ni in whole plant was not enough to be detected. As investigated, the

plant is usually consumed in whole and not in parts. The investigation reveals that Ni toxicity is unlikely to occur with *E. prostrata* at present concentration.

E. prostrata does not seems a suitable candidate for cleaning nickel from contaminated soil because it was not absorbed enough to be detected in plant body.

Tin: As mentioned earlier, Tin was below detecting limit in the entire three soil samples and in whole plant or any of the plant parts. The permissible limit for Sn is 14 mg/day/person (FAO/WHO, 2005). As Sn was below detecting limit in sample, hence there is no risk from *E. prostrata* if consumed.

The BAC value was also zero and therefore the plant is not good extractor for Tin to play environment cleaning role. Furthermore, as the Tin was absent from the soil this necessitates more investigation to grow *E. prostrata* in soil with detectable quantity of Tin for monitoring its environmental role.

Table 12. Toxic heavy metals composition (ppm) of three representative soil samples.

Soil samples	Heavy Metals concentration (ppm) found in 3 soil samples									
	Pb	Cd	Cr	Co	Ag	Hg	As	Mo	Ni	Sn
Footpath	5.20 ± 0.126	3.6 ± 0.076	2.0 ± 0.047	2.2 ± 0.017	2.0 ± 0.060	21.5 ± 0.017	6.31 ± 0.003	1.5 ± 0.019	0.12 ± 0.003	BDL
Stream sides	3.3 ± 0.015	3.2 ± 0.050	1.9 ± 0.050	2.2 ± 0.012	2.3 ± 0.009	15.4 ± 0.019	7.25 ± 0.007	2.0 ± 0.023	0.12 ± 0.010	BDL
Gardens	2.3 ± 0.012	2.5 ± 0.050	2.2 ± 0.033	2.0 ± 0.015	2.0 ± 0.067	10.0 ± 0.050	6.23 ± 0.018	1.6 ± 0.033	0.10 ± 0.033	BDL

Values are the mean of 3 replicates with ± SEM, BDL= below detecting limit

Table 13. Heavy metals composition (ppm) of whole plant and parts of *E. granulata* collected from three soil samples.

Sample analyzed	*Euphorbia granulata* from footpaths									
	Pb	Cd	Cr	Co	Ag	Hg	As	Mo	Ni	Sn
Whole plant	2.7 ± 0.015	BDL	BDL	0.11 ± 0.003	1.8 ± 0.015	29.36 ± 0.056	2.92 ± 0.012	1.9 ± 0.015	0.13 ± 0.003	BDL
Roots	3.9 ± 0.015	3.6 ± 0.015	0.6 ± 0.015	0.12 ± 0.003	2.1 ± 0.05	33.0 ± 0.109	8.5 ± 0.023	2.0 ± 0.054	0.15 ± 0.003	BDL
Stem	2.0 ± 0.039	BDL	BDL	0.11 ± 0.00	1.0 ± 0.022	25.1 ± 0.033	1.87 ± 0.032	1.8 ± 0.015	0.10 ± 0.003	BDL
Leaves	2.5 ± 0.029	BDL	BDL	0.10 ± 0.003	1.3 ± 0.023	24.0 ± 0.186	0.55 ± 0.003	1.9 ± 0.01	0.13 ± 0.003	BDL
Euphorbia granulata from stream sides										
Whole plant	3.5 ± 0.033	BDL	BDL	0.12 ± 0.003	1.4 ± 0.01	20.0 ± 0.109	2.25 ± 0.023	1.8 ± 0.015	0.13 ± 0.009	BDL
Roots	4.1 ± 0.083	3.7 ± 0.033	0.2 ± 0.012	0.14 ± 0.00	1.8 ± 0.015	20.0 ± 0.044	9.43 ± 0.009	1.8 ± 0.007	0.14 ± 0.003	BDL
Stem	2.4 ± 0.026	BDL	BDL	0.12 ± 0.00	1.5 ± 0.015	19.5 ± 0.022	1.13 ± 0.013	1.7 ± 0.027	0.11 ± 0.003	BDL
Leaves	2.2 ± 0.018	BDL	BDL	0.12 ± 0.00	1.1 ± 0.033	18.0 ± 0.058	0.44 ± 0.006	1.8 ± 0.012	0.13 ± 0.003	BDL
Euphorbia granulata from gardens										
Whole plant	2.1 ± 0.012	BDL	BDL	0.10 ± 0.003	1.4 ± 0.021	22.0 ± 0.104	2.39 ± 0.009	1.7 ± 0.021	0.13 ± 0.006	BDL
Roots	2.4 ± 0.012	3.6 ± 0.013	0.2 ± 0.012	0.13 ± 0.00	1.9 ± 0.021	21.5 ± 0.087	8.42 ± 0.012	1.8 ± 0.021	0.14 ± 0.00	BDL
Stem	2.2 ± 0.015	BDL	BDL	0.10 ± 0.003	1.1 ± 0.021	21.0 ± 0.076	1.25 ± 0.025	1.7 ± 0.021	0.11 ± 0.00	BDL
Leaves	2.5 ± 0.01	BDL	BDL	0.10 ± 0.003	1.2 ± 0.015	22.0 ± 0.132	0.45 ± 0.012	1.7 ± 0.012	0.13 ± 0.006	BDL

Values are the mean of 3 replicates with ± SEM, BDL= below detecting limit

Table 14. Bio-accumulation coefficient (BAC) for toxic heavy metals by *E. granulata* calculated from Tables 12 and 13.

Sample analyzed	BAC for whole plant and plant parts from footpaths									
	Pb	Cd	Cr	Co	Ag	Hg	As	Mo	Ni	Sn
W. plant	0.52	0.00	0.00	0.05	0.90	1.37	0.46	1.27	1.08	0.00
Roots	0.75	1.00	0.30	0.05	1.05	1.53	1.35	1.33	1.25	0.00
Stem	0.38	0.00	0.00	0.05	0.50	1.17	0.30	1.20	0.83	0.00
Leaves	0.48	0.00	0.00	0.05	0.65	1.12	0.09	1.27	1.08	0.00
BAC for whole plant and plant parts from stream sides										
W. plant	1.06	0.00	0.00	0.05	0.61	1.30	0.31	0.90	1.08	0.00
Roots	1.24	1.16	0.11	0.06	0.78	1.30	1.3	0.90	1.17	0.00
Stem	0.73	0.00	0.00	0.05	0.65	1.27	0.16	0.85	0.92	0.00
Leaves	0.67	0.00	0.00	0.05	0.48	1.17	0.06	0.90	1.08	0.00
BAC for whole plant and plant parts from gardens										
W. plant	0.91	0.00	0.00	0.05	0.70	2.20	0.38	1.06	1.30	0.00
Roots	1.04	1.44	0.09	0.07	0.95	2.15	1.35	1.13	1.40	0.00
Stem	0.96	0.00	0.00	0.05	0.55	2.10	0.20	1.06	1.10	0.00
Leaves	1.09	0.00	0.00	0.05	0.60	2.20	0.07	1.06	1.30	0.00

Table 15. Daily permissible limit (for ingestion) for toxic heavy metals.

Metal	Daily Permissible Limit (PL)	Recommended by	Reference source
Pb	Up to 0.025 mg	FAO/WHO	FAO/WHO, (2005)
Cd	Up to 0.007 mg	FAO/WHO	FAO/WHO, (2005)
Cr	Up to 0.25 mg	WHO	WHO, (1996)
Co	Not specified	EFSA	EFSA, (2012)
Ag	0.39 mg	EFSA	EFSA, (2005)
Hg	Up to 0.005 mg	FAO/WHO	FAO/WHO, (2005)
As	Up to 0.15 mg	FAO/WHO	FAO/WHO, (2005)
Mo	0.075-0.25 mg	NRC-USA	FNB (1989)
Ni	0.15 mg	EFSA	EFSA, (2006)
Sn	Up to 0.14 mg	FAO/WHO	FAO/WHO, (2005)

WHO = World Health Organization, FAO = Food and Agriculture Organization, NRC-USA = National Research Council USA, NAS = National Academy of Science, EFSA = European Food Safety Authority

Table 16. Possible daily intake (PDI) of toxic heavy metals (60kg person) for *E. granulata.*

PDI (mg/day/person) for whole plant from footpaths										
Heavy metals	Pb	Cd	Cr	Co	Ag	Hg	As	Mo	Ni	Sn
Conc. in dry whole plant (ppm)	2.7 ± 0.026	BDL	BDL	0.11 ± 0.006	1.8 ± 0.026	29.36 ± 0.096	2.92 ± 0.021	1.9 ± 0.026	0.13 ± 0.006	BDL
conc. in fresh whole plant (ppm)	0.45	NA	NA	0.018	0.3	4.86	0.48	0.31	0.02	NA
permissible limit (mg/day/person)	Up to 0.025	Up to 0.007	Up to 0.25	NS	Up to 0.39	Up to 0.005	Upto 0.15	0.075-0.25	Upto 0. 15	Upto 0.14
PDI (mg/day/person)	29.8 (10^{-6})	NA	NA	1.21 (10^{-6})	19.87 (10^{-6})	324.1 (10^{-6})	32.2 (10^{-6})	20.98 (10^{-6})	1.4 (10^{-6})	NA
PDI (mg/day/person) for whole plant from stream sides										
Conc. in dry whole plant (ppm)	3.5 ± 0.057	BDL	BDL	0.12 ± 0.006	1.4 ± 0.017	20.0 ± 0.189	2.25 ± 0.04	1.8 ± 0.026	0.13 ± 0.015	BDL
conc. in fresh whole plant (ppm)	0.58	NA	NA	0.02	0.23	3.3	0.37	0.3	0.021	NA
PDI (mg/day/person)	38.6 (10^{-6})	NA	NA	1.32 (10^{-6})	15.45 (10^{-6})	220 (10^{-6})	24.8 (10^{-6})	19.9 (10^{-6})	1.4 (10^{-6})	NA
PDI (mg/day/person) for whole plant from gardens										
Conc. in dry whole plant (ppm)	2.1 ± 0.02	BDL	BDL	0.10 ± 0.006	1.4 ± 0.021	22.0 ± 0.104	2.39 ± 0.015	1.7 ± 0.021	0.13 ± 0.006	BDL
conc. in fresh whole plant (ppm)	0.35	NA	NA	0.02	0.23	3.64	0.4	0.28	0.021	NA
PDI (mg/day/person)	23.2 (10^{-6})	NA	NA	1.1 (10^{-6})	15.45 (10^{-6})	242 (10^{-6})	26.5 (10^{-6})	18.7 (10^{-6})	1.43 (10^{-6})	NA

Values are the mean of three replicates with ± SEM, NA = not applicable

Table 17. Heavy metals composition (ppm) of whole plant and parts of *E. prostrata* collected from three soil samples.

Sample analyzed	*Euphorbia prostrata* from footpath									
	Pb	Cd	Cr	Co	Ag	Hg	As	Mo	Ni	Sn
Whole plant	3.1 ± 0.029	BDL	BDL	0.13 ± 0.003	1.7 ± 0.050	25.19 ± 0.042	2.4 ± 0.009	2.0 ± 0.056	BDL	BDL
Roots	3.45 ± 0.007	3.0 ± 0.076	2.50 ± 0.023	0.18 ± 0.007	2.2 ± 0.060	29.5 ± 0.060	7.60 ± 0.009	2.0 ± 0.043	0.13 ± 0.003	BDL
Stem	1.93 ± 0.088	BDL	BDL	0.13 ± 0.007	1.2 ± 0.038	20.5 ± 0.033	1.88 ± 0.012	1.5 ± 0.026	BDL	BDL
Leaves	2.53 ± 0.088	BDL	BDL	0.12 ± 0.006	1.3 ± 0.037	22.5 ± 0.060	0.38 ± 0.009	2.3 ± 0.019	BDL	BDL
Euphorbia prostrata from stream sides										
Whole plant	2.5 ± 0.033	BDL	BDL	0.14 ± 0.009	1.5 ± 0.040	20.5 ± 0.035	2.27 ± 0.083	1.9 ± 0.034	BDL	BDL
Roots	2.8 ± 0.020	3.4 ± 0.020	2. 2 ± 0.015	0.16 ± 0.010	2.9 ± 0.018	24.0 ± 0.047	8.15 ± 0.007	2.3 ± 0.037	0.14 ± 0.007	BDL
Stem	2.5 ± 0.023	BDL	BDL	0.11 ± 0.006	1.5 ± 0.017	23.0 ± 0.027	1.28 ± 0.015	1.5 ± 0.027	BDL	BDL
Leaves	2.0 ± 0.018	BDL	BDL	0.11 ± 0.012	2.0 ± 0.030	19.5 ± 0.027	0.31 ± 0.006	2.1 ± 0.043	BDL	BDL
Euphorbia prostrata from garden										
Whole plant	2.2 ± 0.036	BDL	BDL	0.10 ± 0.003	1.5 ± 0.015	14.3 ± 0.033	2.08 ± 0.015	1.6 ± 0.030	BDL	BDL
Roots	2.3 ± 0.015	2.0 ± 0.023	2.2 ± 0.026	0.12 ± 0.010	2.1 ± 0.029	15.0 ± 0.055	7.48 ± 0.015	1.7 ± 0.015	0.12 ± 0.013	BDL
Stem	1.8 ± 0.012	BDL	BDL	0.10 ± 0.007	1.2 ± 0.024	14.0 ± 0.027	1.4 ± 0.009	1.4 ± 0.015	BDL	BDL
Leaves	1.5 ± 0.018	BDL	BDL	0.10 ± 0.003	1.1 ± 0.010	11.0 ± 0.015	0.43 ± 0.015	1.8 ± 0.019	BDL	BDL

Values are the mean of 3 replicates with ± SEM, BDL= below detecting limit,

SEM = standard error of mean

Table 18. Bio-accumulation coefficient (BAC) for toxic heavy metals by *E. prostrata* calculated from Tables 12 and 17.

Sample analyzed	BAC for whole plant and plant parts from footpath									
	Pb	Cd	Cr	Co	Ag	Hg	As	Mo	Ni	Sn
W. plant	0.6	0.0	0.0	0.06	0.85	1.17	0.45	1.33	0.0	0.0
Roots	0.66	0.83	1.25	0.08	1.1	1.37	1.43	1.33	1.08	0.0
Stem	0.37	0.0	0.0	0.06	0.6	0.95	0.35	1.0	0.0	0.0
Leaves	0.49	0.0	0.0	0.05	0.65	1.05	0.07	1.53	0.0	0.0
BAC for whole plant and plant parts from stream sides										
W. plant	0.76	0.0	0.0	0.06	0.65	1.33	0.44	0.95	0.0	0.0
Roots	0.85	1.06	1.16	0.07	1.26	1.56	1.57	1.15	1.17	0.0
Stem	0.76	0.0	0.0	0.05	0.65	1.49	0.25	0.75	0.0	0.0
Leaves	0.61	0.0	0.0	0.05	0.87	1.27	0.06	1.05	0.0	0.0
BAC for whole plant and plant parts from gardens										
W. plant	0.96	0.0	0.0	0.05	0.75	1.43	0.49	1.0	0.0	0.0
Roots	1.0	0.8	1.0	0.06	1.05	1.5	1.77	1.06	1.2	0.0
Stem	0.78	0.0	0.0	0.05	0.6	1.4	0.33	0.88	0.0	0.0
Leaves	0.65	0.0	0.0	0.05	0.55	1.1	0.1	1.13	0.0	0.0

Table 19. Possible daily intake (PDI) of toxic heavy metals (60kg person) for *E. prostrata*.

PDI (mg/day/person) for whole plant from footpath										
Heavy metals	Pb	Cd	Cr	Co	Ag	Hg	As	Mo	Ni	Sn
Conc. in dry whole plant (ppm)	3.1 ± 0.029	BDL	BDL	0.13 ± 0.003	1.7 ± 0.050	25.19 ± 0.042	2.4 ± 0.009	2.0 ± 0.056	BDL	BDL
Conc. in fresh whole plant (ppm)	0.51	NA	NA	0.02	0.28	4.17	0.4	0.33	NA	NA
permissible limit (mg/day/person)	Up to 0.25	Up to 0.07	0.05-0.20	0.15-0.5	Up to 0.01	Up to 1.0	Up to 150	80-250	Up to 0. 50	Up to 14000
PDI (mg/day/person)	34.2 (10^{-6})	NA	NA	1.43 (10^{-6})	18.77 (10^{-6})	278 (10^{-6})	26.5 (10^{-6})	22.0 (10^{-6})	NA	NA
PDI (mg/day/person) for whole plant from stream sides										
Conc. in dry whole plant (ppm)	2.5 ± 0.033	BDL	BDL	0.14 ± 0.009	1.5 ± 0.040	20.5 ± 0.035	2.27 ± 0.083	1.9 ± 0.034	BDL	BDL
Conc. in fresh whole plant (ppm)	0.41	0.0	0.0	0.02	0.25	3.4	0.38	0.31	NA	NA
PDI (mg/day/person)	27.6 (10-6)	NA	NA	1.55 (10-6)	16.56 (10-6)	226.3 (10-6)	25.1 (10-6)	20.98 (10-6)	NA	NA
PDI (mg/day/person) for whole plant from gardens										
Conc. in dry whole plant (ppm)	2.2 ± 0.036	BDL	BDL	0.10 ± 0.003	1.5 ± 0.015	14.3 ± 0.033	2.08 ± 0.015	1.6 ± 0.030	BDL	BDL
Conc. in fresh whole plant (ppm)	0.37	0.0	0.0	0.017	0.25	2.37	0.35	0.27	NA	NA
PDI (mg/day/person)	24.29 (10-6)	NA	NA	1.1 (10-6)	16.56 (10-6)	57.87 (10-6)	23.0 (10-6)	17.66 (10-6)	NA	NA

NA = not applicable, SS1 = soil sample 1, SS2 = soil sample 2, SS3 = soil sample 3

5. Antidiabetic activity

Euphorbia granulata Forssk

For this experiment two sets of rabbit were used; i.e. Set 1 (normal) and 2 (diabetic). Each set comprised of 3 groups' i.e. negative control, positive control and test groups. For both sets the changes in blood glucose level (BGL) with standard drug (glibenclemide), 200 mg test extract and in negative control are summarized in Table 20. The results are discussed separately for the sets of rabbits and then each set was compared other.

Comparison of three normal groups: Group 1 was given only empty capsule shell and blood level monitored at different time intervals. During 72 hours after the capsule feeding no change in BGL occurred. The group that received glibenclemide showed a significant ($p<0.01$) drop in BGL (20.4%) compared to the base time level. In this group the BGL then returned rapidly to the base line level (103mg ± 5.788) after 24 hours and maintained till 72 hours. With 200mg of ethanolic plant extract the BGL decreased insignificantly ($p>0.05$) from 101 (± 5.612) to 96 (± 6.245) mg by 6.9% compared base line level. The lower level of 96 mg was then maintained till 48 hours. The base line level of 103mg (±6.87) was re-attained at 72 hours. Rapid return to normal with reference drug and low normalization with extract dose shows that the extract effect was long acting than the standard drug. Although the decrease in BGL was not significant (>0.05) with plant extract but this small effect was produced with crude extract and optimistically significant decrease can be achieved with isolated active principals. Diabetes is one of the challenging areas to the medical profession. The disease is characterized by high blood glucose level due to complete absence or decrease secretion of insulin from β-cells of pancreas (Sunil, 2009; Priyanka *et al*., 2010). Due to extensive use of insulin the patient of diabetes are becoming less responsive to the use of insulin (Sokeng *et al*., 2007; Selvan *et al*., 2008). This necessitates an alternative safe and effective treatment (Rekha *et al*., 2010; Wadood *et al.*, 2007). Before the preparation of insulin 1921 the herbal extracts were used for the treating diabetes (El-Shenawy *et al*., 2006). *E. granulata* might have increased the insulin secretion or might have insulin like effect by itself.

Comparison of three diabetic groups: Group 4 was diabetic negative control and was given only empty capsule shell. This group sustained the high glucose level throughout the experimental period' i.e. 222 mg (± 8.989) at zero time to 222 mg (± 8.591) at end. Group 5 comprised diabetic induced rabbits. In this group the BGL dropped significantly ($p<0.001$) from 222 mg (± 8.927) at zero time to 194mg (±8.081) at 8 hours. Unlike group 2 here the BGL rose again after 8 hours to 218mg (± 9.182) at 24 hours, very close value to the zero time level. The zero time level of 224mg (± 8.689) was touched after 72 hours. In this group with standard drug the BGL level was decrease 12.6%. Group 6 received 200mg/kg of plant extract. Here BGL dropped by 3.4% from initial 212mg (± 13.864) to final level of 205mg (± 13.58). This level of 205mg (± 13.372) prevails till 24 hours and rose to 209mg (± 11.41) at 48 hours. In this group the BGL never reached the base line level of 212mg. The results suggest that the plant have some active principals that help reducing the BGL.

Comparison of three diabetic with three normal groups: Group 1 and 4 (both negative controls) were found with no change in the BGL. This proves that the experimental environments were good and there was no interfering factor affecting BGL. Both groups 2 and 5 were treated with standard drugs and both were noted with almost identical results. The difference between the two groups was that the lowest BGL in group 2 was achieved and maintained till 24 hours. Here the base line was re-attained at 72 hours. However, in group 5 the BGL dropped from 222 (± 8.927) to 194mg (± 8.081) at 8 hours and then rose to 218mg (± 9.182) at 24 hour. This level (219mg ± 9.566) was maintained till 48 hours. The base line level was touched only after 72 hours. The possible explanation to this unusual effect may be due to slow metabolization of the drug in already metabolically stressed liver of the diabetic rabbits. There may also be decrease secretion of drug in induced diabetic rabbits. In this group the decrease in BGL level was only 12.6% compared to 20.4% with normal rabbits. Sulphonylureas drugs stimulate insulin secretion from β-cells (Wadood *et al.*, 2007). In induced diabetic rabbits most of these cells are damaged (Sokeng *et al*., 2007), therefore less chances of insulin secretion in this group. Therefore, the drug was less effective in diabetic than in the normal rabbits. Lastly comparing group 3 with 6 (both tested groups). In group 3 the BGL had decreased

6.9% while in group 6 the decrease was 3.4%. The normal physiology and normal β-cells in group 3 might have influenced the large decrease in BGL in this group. In both groups the decrease in BGL was not significant ($p>0.05$). Statistically none significant however the overall decrease of 5.2% and 3.4% in these two groups compared to negative controls suggests the hypoglycemic potential of the plant. As this small effect was noted with crude extract, hopefully the purified active principals may control the BGL significantly.

It was noticed that the plant extract has weak hypoglycemic activity. These effects were more pronounced in normal rabbits than in diabetics.

***Euphorbai prostrata* Aiton**

The above grouping pattern was also repeated for *Euphorbia prostrata*. The findings for this plant extract are presented in Table 21. In the present experiment the results obtained will be compared not only among themselves but also with those obtained with *E. granulata*.

Comparison of three normal groups: Group 1 does not receive any drug and was treated as negative control. BGL for this group was monitored like treated groups at different time intervals. The results reveal that the BGL of this group remained unchanged. It ranged between 83 to 84 mgs only. BGL of group 2 was monitored for the effects of glibenclemide. In this group significant ($p<0.01$) drop was noted from 90mg (± 7.635) to 68mg (± 6.979) at 8 hours of drug administration (24.4% decrease). As happened for *E. granulata* here also the BGL normalized to base line level 0f 90mg (± 8.012) after 24 hours. This indicates that soon after absorption, the standard drug activated the secretion of insulin which lowers BGL. Thereafter it was rapidly deactivate or secreted. Group 3 received 200mg of plant extracts. For this group, initially the BGL did not change till 2 hours. After 2 hours the BGL began to decrease significantly ($p<0.01$) from 101mg (± 5.523) to 98mg (± 5.586) till 8 hours. This was a 3% decrease compared to zero time level. The two hour delay in reducing the BGL might be due to slow absorption from gut or slow onset of action. At 72 hours the BGL re-attain the base line level. The significant decrease in BGL with plant extracts support the traditional antidiabetic use of plant.

Comparison of three diabetic groups: this set of rabbits consisted of alloxan induced diabetic groups. Group 4 was not treated and was kept as negative control. Slight and insignificant variation was observed in BGL for group 4 (Table 21). The variation in BGL ranged between 223 and 224mg. Group 5 was treated as positive control. As for group 2 here also the BGL dropped from 223mg (± 11.696) at zero time to 193mg (± 8.264) i.e. a 13.5% decrease. This was a significant ($p<0.01$) decrease compared to negative control. The group 6 was treated 200mg of plant extract. In group 6 the BGL dropping effect was again delayed 1 hour compared to standard drug. This delay was most probably because of slow absorption of extract or slow mode of action. The overall BGL dropped by 3.6% from 221mg (± 14.87) to 213mg (± 14.4) at 24 hours. Although statistical results shows that drop of BGL for this group is not significant however comparing the 0.0% decrease in negative control it can be stated optimistically that the extract have some week antidiabetic activities. Furthermore, looking at the results carefully reveal that the BGL remain below the normal (for this group) even after 72 hours (217mg ± 14.41). The trend of reducing BGL suggests that the extract might have some active principals. This control over BGL might be due to enhancing insulin seretion, repairing or regenerating properties for β-cells or might be due to antioxidant potential of the extract (Khanam & Dewan, 2008; Rekha *et al*., 2010).

Comparison of three diabetic with three normal groups: Normal healthy rabbits contain normal β-cells with normal insulin secretion. By injecting alloxan, 46% of the β-cells of are damaged. These rabbits then become unable to secret the required amount of insulin for glucose metabolism (Sokeng *et al*., 2007; Khanam & Dewan, 2008). In the above 2 sets of rabbits, group 1 was negative control for normal rabbits while group 4 was for diabetic groups. Both these groups were found with a constant BGL during the experimental period. This indicated that in absence of stimuli, both groups of rabbits maintained the physiological levels. Group 2 was positive control for normal set of rabbits while group 5 normal for diabetic set. In both cases the blood glucose level dropped but differently. Comparatively larger decrease (24.4%) was noted in normal rabbits compared to diabetics (13.5%). The possible reason is that healthy rabbits have normal number of β-cells and have more potential to secret insulin (Abu-

zaiton, 2009). The diabetic induced rabbits have small number of β-cells and therefore secrete small amount of insulins for glucose metabolism. Comparing the test group of normal rabbits (group 3) with that of test group for diabetic rabbits (Group 6), it was revealed that significant ($p<0.01$) decrease in BGL was occurred in the first case than in the second ($p>0.05$). This again can be explaining on the basis of differences in the number of β-cells in the two groups.

The overall results for both plants show that *E. granulata* induces hypoglycemic effects earlier than *E. prostrata*. Both the plants have potential hypoglycemic effects. More effect was noted for normal rabbits than in alloxan treated diabetic rabbits.

6. Antilipidemic activity

***Euphorbia granulata* Forssk**

To induce disturbed lipid profile rabbits were treated with isoprenaline. Table 22 compares the lipid levels of rabbits before and after treatment with isoprenaline. In the present study the rabbits were divided into 10 groups. Group 1, 2 and 3 comprised of normal healthy rabbits and were treated as negative control, positive control and test groups respectively. Table 23 summarizes the impact of different treatments on lipid profile in these groups. Group 4, 5 and 6 comprised of isoprenaline treated rabbits and were treated in similar way as above. Table 24 is set with the effects of different treatments in these groups. Goup 7, 8 and 9 were diabetic rabbits with already disturbed lipid levels the results for these goups are presented in Table 25. Table 26 shows the results in preventive control group 10.

To evaluate the lipid lowering potential of *E. granulata* six variables were monitored. These variables includes total cholesterol (TC), high density lipids (HDL), low density lipids (LDL), very low density lipids (VLDL), triglycerides (TG) and free fatty acids (FFA).

Lipid profile of induced myocardial infarcted rabbits: With isoprenaline treatment of group 4, TC level increased significantly ($p < 0.01$) from 45 (± 7.28) to 81 (± 14.7), HDL from 29 (± 5.31) to 36 (± 4.301), LDL from 27 (± 4.658) to 36 (± 4.97), VLDL from 21 (± 3.701) to 32 (± 4.438), TG from 47 (± 6.181) to 74 (± 7.106) and FFA

from 57 (± 3.347) to 70 (± 4.583) mg/dl. Similar increases also occurred in group 5 and 6.

Comparison of normal rabbits: As group 1 was not treated with any drug, this group maintained a constant level of lipids for 3 days of experiment (Table 23). Resveratrol is known to protect against atherosclerosis by inhibiting vascular cells adhesion molecule 1 (VCAM-1), intracellular adhesion molecule 1(ICAM-1) and transcription factor (NF-I) (Castro *et al*., 2009). In the present study resveratrol was used to check the affect in group 2 rabbits while the impacts of extracts were noted in group 3. After three days of treatment resveratrol, the TC level significantly ($p<0.01$) dropped by 14% from 50 mg (± 6.017) to 43 mg (± 5.339). With 200 mg extracts of *Euphorbia granulata*, the TC dropped by 11.7% from 59 (± 5.523) to 52 (± 8.585) mg. The values of LDL decreased by 17.2% from 29 (± 4.528) to 24 mg (± 3.317) in positive control group, while the decrease in experimental group was 8.6% from 35 (±3.493) to 32 (±2.881) mg/dl. TG decreased by 9% from 44 (± 3.674) to 40 mg (± 2.608) in positive control while no decreased noted in test group. Similarly FFA decreased by 10.5% from 57 (± 5.07) to 51 mg (± 4.278) however the levels had not changed with plant extracts. No change was noted in HDL either with resveratrol or plant extract. The values of VLDL remained unchanged with positive control but was decreased by 14.3% from 21 (± 4.658) to 18 mg (± 3.674)/dl.

Researchers have pointed that saponins and flavonoids exerts hypolipidemic effect and protect against cardiac diseases (Khursheed *et al*., 2010; Farsam *et al*., 2003). Table 6 shows that *E. granulata* contains good amount of flavonoids (0.7 ± 0.02 mg/gm) and saponins (0.2 ± 0.0). The decrease in TC, LDL and VLDL with plant extract may be due to the presence of these natural products. The extract may have exerted this affect by decreasing the absorption and digestion of different lipids or may have inhibited the important enzymes in liver responsible for the synthesis of TC, LDL and VLDL. Another possibility is the activation of cholesterol-7-alpha-hydroxylase responsible for conversion of cholesterol to bile acid (Gaamoussi *et al*., 2010). Hormone sensitive lipoprotein lipase causes the breaking down of TG (Jarald *et al*., 2009). No change in TG and FFA indicate that the extract did not activate or suppress the activity of this enzyme.

Comparison of induced myocardial infarcted rabbits: Atherosclerosis has complex many events aetiology mainly initiated by the oxidation of lipoprotein (Choi *et al*., 2010). Isoprenaline induces myocardial infarction in animals having identical metabolic and morphologic aberration to that produced in human being (Ganesan *et al*., 2007). The major changes are brought about by necrosis and damage of myocardial membrane (Sivakumar *et al*., 2007). In this set of rabbits group 4 was treated as negative control, group 5 as positive control while groups 6 as test group. Although slight variation was noted in negative control group during 3 days experimental period but over all the changes were negligible compare to positive and test groups. Group 5 was treated with resveratrol and group 6 with 200 mg/kg of extracts. In positive control the TC decreased by 8% from 87 (± 13.73) to 80 (± 13.44) mg, while in test group the decrease was 7.2% from 83 (± 9.487) to 77 (± 9.45) mg/dl. This decrease is almost equal in both these groups. The levels of LDL decreased by 11.1% from 36 (± 3.701) to 32 (± 4.336) while the decrease in test group was 7.9% from 38 (± 3.962) to 35 (± 3.899). This shows that the control drug was more effective than plant extract. The levels of VLDL decreased by 11.5% from 26 (± 3.24) to 23 (± 3.209) mg/dl compared to 16.7 % decrease with test extract from 30 (± 3.962) to 25 (± 3.701). In case of VLDL the extract was more effective than the positive control. Table 24 shows that values of HDL and TG changed neither with standard drug nor with test extracts. It was further noted that in positive control group, the FFA levels significantly ($p<0.05$) decreased by 9.5% from 63 (± 3.24) to 57 (± 3.209) mg/dl compared to test extract where no change was noted. The non-significant reduction in lipid profile with standard drug might be due to the fact that the damage with isoprenaline and the pathological process might still be continued and be more than the curative effects of the drug. Comparing the changes in TG, LDL and VLDL it seems that the extract was highly effective in decreasing the lipid level in rabbits. The present affects with plant were achieved with cruide extract; this reduction can be more significant if the active principals be used in pure form. Secondly this improvement occurred when the damaging activities of isoprenaline were also occurring in the body. Optimistically it can be stated that the extracts had the ability to lower the level of TC, LDL and VLDL with possibly any of the mechanism described above.

Comparison of diabetic rabbits: Reports show that induced diabetes in animals disturbs the normal lipid profile (Rajasekaran *et al*., 2006). In diabetes the high BGL and reduced insulin in diabetics stimulate hormone sensitive lipases (HSL). HSL results in breaking down of TG which further leads to high FFA levels. The high levels of FFA lead to the synthesis of phospholipids, TC, LDL, VLDL and TG (Jarald *et al*., 2009; Rajasekaran *et al*., 2006).The present study was focused on evaluating the lipid lowering ability of *Euphorbia granulata* in diabetes rabbits. In groups 7, 8 and 9 the rabbits were diabetic and the lipid levels were already deranged. The negative control group had no change in the overall lipid profile except FFA (Table 25). This decrease in FFA level was 5.8% from 69 (± 2.588) to 65 (± 3.834). This decrease in FFA even in the absence of any external stimuli was unexpected but suggests the possible regeneration of β-cells. The increase in β-cells may have enhanced insulin level and have decrease HSL release (Khanam & Dewan. 2008). With 3 days treatment the levels of TC decreased significantly ($p<0.01$) by 11.6% from 69 (± 7.396) to 61 (± 5.63) in positive control group. In extract treated group this decrease was 6.7% from 75 (± 3.271) to 70 (± 3.962). The decrease with crude extract was comparatively small but hopefully the purified principal might be more affective that the extract. Treatment with 200 mg plant extract also reduced the levels of LDL by 7.1% from 42 (± 2.55) to 39 (± 1.581) mg/dl ($p<0.01$) compared to positive control of 13.9% from 36 (± 4.301) to 31 (± 3.0). VLDL dropped by 17.4% from 23 (± 3.962) to 19 (± 3.674) with plant extract and only 13.6% from 22 (± 4.97) to 19 (± 4.506). The results indicate that plant extract is more affective in VLDL compared to standard drug. Furthermore FFA levels decrease by 7.1% from 70 (± 3.347) to 65 (± 2.702) mg /dl with standard drug but reverse effect was noted with extract. The level of FFA increased by 4.3% from 69 (± 2.55) to 72 (± 2.28) mg / dl. This increase was found unexpected. The level of HDL and TG were not affected either with standard drug or plant extract. The data obtained in this study support the antilipidemic use of the extract. In diabetic rabbits the lipid normalizing potential is either by insulin like mechanism or increasing insulin secretion from β-cells. It may also be due to regeneration of β-cells or normalization of damaged cells. The extract may also by

decreasing absorption and digestion or by stimulating and inhibiting enzymes involved in lipid metabolism.

Comparison of preventive groups: Group 10 was preventive control and received 200 mg plant extract before and along with administration of isoprenaline. Table 22 compares the levels of lipid profile for group 10 and groups 4, 5 and 6. It is very clear that treatment of normal rabbits with isoprnaline produced significant changes in group 4, 5 and 6. In preventive control group the changes in lipid profile were negligible. Isoprenaline induced myocardial necrosis is a combined effect of increased cAMP production alongside lipid accumulation, lipid peroxidation and free radical generation (Ganesan *et al*., 2007). The extract might have the constituents that may inhibit adenylate cyclase or decrease its activity and in turn reduces lipid accumulation by myocardium. Analysis of the plant for phytochemicals was found with good quantity of phenolic compounds and saponins. These compounds have excellent free radical scavenging and antihyperlipidaemic activity (Rajasekaran *et al*., 2006). The free radical scavenging might also have protected the myocardium from the damage in this group.

Comparison of the above four situations: In the above sets of rabbits group 1, 4 and 7 were negative control groups. Group 1 was with normal β-cells physiology. In this group no change occurred in the lipid profile during the experimental period. Group 4 was with myocardial infarction. In this group also the lipid levels were maintained. Group 7 rabbits were with damaged β-cells. Here all the parameters of lipid profile remain constant except FFA which showed decrease. The possible cause may be natural regeneration process of β-cells. Group 10 maintained the normal level even after the induction of isoprenaline. The possible cause may be the presence of natural antioxidants in the plant extract. These antioxidants may have prevented the injurious effect of free radicals generated during the degenerative process of myocardial cells. Group 2, 5 and 8 received treatment with resveratrol (positive controls). Resveratrol is an important antioxidant which protects myocardium by its anti-inflammatory, antithrombotic, anti-proliferative and antilipidemic properties (Castro *et al*., 2009). In group 2 and 8 resveratrol effectively decreased TC, LDL, TG and FFA. In group 5 it reduced the levels of TC, LDL, VLDL and FFA but had no effect on TG. Group 3, 6 and 9 were treated with plant extract. In

all three groups of rabbits the extract reduced the levels of TC, LDL and VLDL but had no effect on the levels of HDL, TG or FFA. This indicates that the extract may have reduced absorption. Another possible reason may have inhibited hydroxyl methyl glutaryl CoA reductase (an enzyme involved in cholesterol synthesis) or may have stimulated cholesterol-7-alpha-hydroxylase (enzyme converting cholesterol into bile acid) (Rajasekaran *et al*., 2006).

The overall results show the suppressive effect of *E. granulata* extracts on TC, LDL and VLDL. This makes the plant a suitable candidate for further study of isolation and identification of the specific constituents responsible for the cardio-protective effect.

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Table 27 summarizes the impact of isoprenaline on lipid profile of rabbits. Here in group 4 and 5 TC, LDL, VLDL, TG and FFA increased with isoprenaline but HDL decreased. Table 28 describes impact of drug and extract on lipid profile of normal rabbits. Table 29 shows variation in lipid levels of the myocardially infracted rabbits with drugs and extract. Results for diabetic rabbits are arranged in Table 30. Group 10 is preventive control group (Table 31).

Both *E. granulata* and *E. prostrata* are used for different ailments without distinction by the local population. The present study is aimed to study and compare the two plants in different situations. Therefore, evaluating *E. granulata* for antilipidemic potential *E. prostrata* was also to compare on the same grounds. The major cause behind cardiovascular diseases is high levels of total lipids, lipoprotein, cholesterol and triglycerides. Sustained high levels ultimately leads to obesity, atherosclerosis (Javed *et al*., 2006), myocardial infarction and brain vascular accidents (Castro *et al*., 2009). Myocardial infarction is reported to be the major health concern and cause of death globally (Khursheed *et al*., 2010). Excessive eating and diabetes contribute to high lipid levels (Moreno *et al*., 2003; Rekha *et al*., 2010). Isoprenaline induced myocardial also derange the normal levels (Ganesan *et al*., 2007). The present study was carried out on normal, induced myocardial infarction and diabetic rabbits. Each set of rabbits were further grouped into negative, positive controls and test rabbits.

Comparison of normal rabbits: In group 1 the levels of lipids sustained throughout the experiment while group 2 and 3showed variation in the profile. The levels of TC, LDL, VLDL and FFA significantly ($p<0.05$) dropped. The levels of TC in extract treated group decreased by 10.4% from 48 (± 4.817) to 43(± 3.674) was less compared to resveratrol treated group where TC decreased by 14.6% from 41 (± 3.564) to 35(± 2.915). LDL decreased by 15.4% from 26 (± 6.181) to 22(± 5.32) with standard treatment but were not affected with treatment of extract. This means that the plant is not effective to control level of LDL. VLDL decreased by 22.2% from 18 (± 4.301) to 14 (± 2.95) mg/dl with extract but only 17.6% from 17 (± 4.583) to 14(± 2.702) with standard treatment. This suggests that, like the experiment with *E. granulata* the present extract was also more effective in mitigating VLDL than standard drug. VLDL is lipoprotein produced by the liver from cholesterol, TG and apolipoprotein. They help transport fats and cholesterol in blood stream and are converted to LDL there. Hence the decrease of VLDL with *E. granulata* extract is an extra benefit not produced with *E. prostrata*. Other lipid parameters remained the same in results of both plants. HDL increased by 6.9% from 29 (± 6.042) to 31 (± 4.817) mg/dl with plant extract but not with standard treatment. This again is an additional benefit meet only with extract. HDL helps removing cholesterol from within the arterial wall and carries them to liver for re-utilization or excretion. People having high level of HDL are less prone to coronary diseases compared to those with low level. This is why they are called good cholesterol.TG changed neither with standard drug nor with plant extract however, FFA decreased by 9.8% from 61 (± 3.493) to 55 (± 2.881) mg/dl but had not responded with plant extract. The levels of both TG and FFA were also not effected in previous study with plant extract from *E. granulata*. This correlates with identical chemical constituents of the two plants which are proved during phytochemical analysis of the two plants (Tables 6 and 7).

Comparison of induced myocardial infarcted rabbits: In group 4 (negative control) all the lipid levels sustained during the experimental 3 day period. Treatment with drug resulted in only 7.6% decrease of TC values from 66 (± 9.354) to 61(± 8.562) compared to 10.9% decrease from 64 (± 4.95) to 57 (± 5.148) with *E. prostrata* extract. Compared to negative control and drug treated rabbits the 10.9% with decrease in TC

with extract is an indication of the plant role in levering blood cholesterol. At day 3 the levels of LDL decreased by by 9.1% from 33 (± 5.675) to 30 (± 4.848) with plant extract compared to 12.9% from 31 (± 4.604) to 27(± 4.712) with standard drug. In experiment with *E. granulata* similar small decrease with extract and large decrease with standard drug was noted. This can be correlates to the almost similar phytochemical constituents of the two plants. A similar decrease in VLDL values from 26 (± 3.082) to 20 (± 3.347) mg/dl with *E. prostrata* extract and from 22 (± 4.743) to 19 (± 3.578) with positive control was also noted. These decreases were 23% with extract and 13.6% with drug, indicates the comparative efficacy of extract. FFA levels decrease by 7.6% from 66 (± 4.025) to 61 (± 3.701) mg/dl in positive control group but had not changed with extract. HDL and TG and FFA neither changed with drug nor with extract. These comparative results show better activity of *E. granulata* in case of VLDL but good control of *E. prostrata* incase of TC and LDL. This difference of results can be correlates to the different combinations of the phytochemicals found in the two plants (Tables 6 and 7).

Comparison of diabetic rabbits: Group 7 was negative control group of diabetic rabbits. The lipid levels here were monitored for three days and were found sustained throughout. Group 8 was positive control. In this group the values of TC decreased by 7.1% from 70 (± 6.979) to 65(± 6.542), LDL by 16.1% from 31 (± 5.385) to 26(± 3.391). The HDL and TG levels had not changed in these rabbits. However the values of VLDL decreased by 17.6% from 17 (± 2.168) to 14 (± 1.517) and FFA by 6.6% from 76 (± 4.743) to 71 (± 5.916) mg/dl with 3days treatment. Group 9 was tested with plant extract. In this group only the levels of VLDL showed a decrease of 15.8% from 19 (± 4.637) to 16 (± 3.536) mg/dl. The levels of other 5 variables had not changed. Comparing lipid lowering potential of the two plants in diabetic rabbits shows few discrepancies. In case of *E. granulata* the levels of FFA decreased even in negative control from 69 to 65 mg/dl however in case of *E. prostrata* the levels of all lipids remained almost constant during the experiment. The standard drug (resveratrol) produces similar results in both experiments. In test rabbits *E. granulata* had effectively controlled and decreased the levels of TC from 75 to 70 (7.1%). Similarly LDL decreased from 42 to 39 (7.1%) and VLDL from 23 to 19 (17.4%). In *E. prostrata* only VLDL decrease from 19 to 16 mg/dl

(15.8%). This further indicates the effectiveness of *E. granulata* in lowering the lipid levels in diabetic rabbits. As already mentioned the presence of good amount of alkaloids and phenolic compounds may be one of the reasons for antilipidemic effect in diabetic rabbits (Adebayo *et al*., 2009).

Comparison of preventive groups of rabbits: In this group also only VLDL decreased by 12.5% from 16 (± 4.438) to 14 (± 4.025) mg/dl while the rest of the lipid profile did not showed change with extract dose. The reason in this group most probably is that the levels of lipids were already within the normal range. Both *E. granulata* and *E. prostrata* have indicated effectiveness in preventing atherosclerosis and in minimizing the damage to myocardial membrane (Tables 22 and 27) in preventive groups.

The above results conclude that both *E. granulata* and *E. prostrata* not only selectively reduces the levels of TC, LDL, VLDL and FFA but also protect the myocardial cells injury induced by free radicals.

7. Hepatoprotective activity

***Euphorbia granulata* Forssk**

Seven groups of experimental rabbits were used in this study. The toxicity effects of CCl_4 on rabbits are presented in Table 32. The results for normal rabbits treated with empty capsule shells, silymarin and plant extract for three days are shown in Table 33. The results of the hepatic damaged rabbits (group 4, 5 and 6) treated with silymarin and plant extracts are tabulated in Table 34. Group 7 was preventive control and the effects on the liver health indicators are arranged in Table 35.

In the present study the liver health was evaluated from six biochemical markers including ALT, AST, bilirubin, urea, albumin and total protein. The values of these variables indirectly reflect the behaviors of liver health (Murugaian *et al*., 2008; Saleem *et al*., 2008; Obianime & Uche. 2008).

Impact of CCl_4 on liver health: Liver is exposed to a number of xenobiotics and is involved in detoxification and metabolism of foreign agents (Prakash *et al*., 2008). A xenobiotic is a strange foreign agent, like drugs and poisons causing liver injury if taken in large amount (Oduola *et al*., 2010). However, some can induce injury at therapeutic

dose (Izunya *et al*., 2010). In the present study CCl_4 was used as a xenobiotic to induce liver injury. Table 32 reveals that liver health indicators deranged in group 4, 5 and 6 rabbits, after treatment with CCl_4. It is evident that the levels of bilirubin increased by105%, 88% and 107% in groups 4, 5 and 6, respectively. Similarly, ALT enhanced by 350%, 322% and 288%, while AST by 220%, 113% and 140% in three groups, respectively. The level of urea decreased to 55, 66 and 62%, respectively with CCl_4 treatment. Albumin dropped 40, 47 and 56% while levels of total protein decreased by 29, 42 and 35% after the treatment. In preventive control group these changes were just 13% (Bn), 23.7% (urea), 41% (ALT), 73% (AST), 20.7% (Alb) and 24% (TP) after CCl_4 treatment. Many researchers noticed gross cytological and anatomical liver changes (Rao *et al*., 2005; Ahsan *et al*., 2009) after CCl_4 induction.

Comparison of normal rabbits: Group 1rabbits were negative control group. Group 2 rabbits were treated with silymarin and group 3 with plant extracts. In group 1 the liver health markers showed negligible changes during the experimental time. This small up and down most probably occurred due to routine wear and tear in the body. As the liver health markers were already within the normal range in group 2 rabbits, therefore treatment with silymarin did not produce any significant change. Only the level of TP slightly increased (3.3%) from 61 (± 4.77) to 63 (± 3.162) gm/dl. In group 3 also, the liver health markers were within the normal range therefore extract produced no change. In this group the level of urea slightly decreased (8.7%) from 23 (± 3.674) to 21mg (± 4.301)/dl. As silymarin exert its effect on injured hepatocytes to restore its normal activity therefore no improvement was made in normal rabbit's hepatocytes except a slight increase in urea production. As urea formation is a normal liver function (Nozad *et al*., 2012), the slight increase in urea levels may be due to increased efficiency of the existing hepatocytes.

Comparison of CCl_4 treated rabbits: Carbon tetrachloride induces liver injury in rats by lipid peroxidation, decrease in antioxidant enzymes activities and excessive production of free radicals (Aghela *et al*., 2007). Thioacetamide metabolism in rats produced reactive oxygen species (ROS) that caused liver cirrhosis (Ahmed *et al*., 2008). Therefore the excessive production of ROS may cause cirrhosis (Iniaghe *et al*., 2008).

Group 4, 5 and 6 were liver damaged rabbits with deranged liver indicator profile. In this set of rabbits, group 4 was not treated with any drug but surprisingly changes were noted in liver health markers during analysis. Slight decrease in urea levels from 6.7 to 6 and increase in Bn values from 7 (± 0.797) to 7.5 (± 0.539) was noted. Alb levels also decreased from 17.5 (± 3.84) to 14.5 (± 3.84) and TP from 42.5 (± 5.85) to 40 (± 5.099). These changes in untreated group most probably indicated that the destructive process induced by CCl_4 in hepatocytes still continued. In silymarin treated group the Bn value decreased by11% from 8.2 (± 0.495) to 7.3 (± 0.458) but Bn value was not affected with extract dose. Bilirubin is a byproduct of heme metabolism in liver. High level indicates problem with liver and bile duct drainage (Saleem *et al*., 2008) and vice versa. ALT and AST are found in cytoplasm of hepatocytes. High levels in blood reflect damage to structural integrity of liver (Ahsan *et al*., 2009). ALT significantly decreased (20%) from 248 (± 13.32) to 198 (± 14.85) and AST (15.65%) from 115 (± 11.2) to 97 (± 8.602) with 3 days extract dose. Similar treatment with silymarin reduced serum ALT by16.3% from 245 (± 13.28) to 205 (± 9.354) and AST by 29.2% from 130 (± 16.1) to 92(± 16.56). This comparable decrease in ALT and AST values with standard drug and extract indicate that plant extract was helpful in repairing the function of liver cells. With 3 days administration of plant extract the levels of urea slightly increased (8.3%) from 7.2 (± 0.914) to 7.8 (± 1.03). Urea level with silymarin increased by 47.5% from 6.1 (± 0.886) to 9 (± 0.667). Urea is produced in the liver and any damage of liver will results in reduced production of urea (Nozad *et al*., 2012). The standard drug was much effective than the plant extract in normalizing the urea formation activity of liver. However, the extract was in unrefined form and hopefully the isolated active principal be more effective than crude extract. Albumin is the major blood protein produced by the liver. High or low levels of albumin directly affect total blood protein and decrease level indicates liver necrosis (Nuhu & Aliyu. 2008). Treatment of rabbits with plant extract enhanced the levels of both Alb and TP. Alb clearly increased by 25% from 14 (± 3.26) to 17.5 (± 3.35) and TP by 20.4% from 44 (± 6.285) to 53 (± 5.477). The results obtained with plant extract were in comparable range to silymarin where the Alb level improved

by 23.5% from 17 (± 3.386) to 21 (± 3.391) and TP by19.1% from 38 (± 2.739) to 47 (± 2.55).

These results are indicative of the potential liver health improvement property of these plant extracts. Studies on histopathology of liver noticed that after normalization of biochemical markers the anatomical and cytological architecture of liver also improved (Rao *et al*., 2005; Ahsan *et al*., 2009).

Comparison of preventive groups: Treating rabbits with CCL_4 without plant extract showed high level changes in liver health markers. Preventive group was given 200 mg/kg of extract before treatment. The results (Table 32) showed small variation in liver health markers values. Comparing the increase that occurred in group 4 (treated) with group 7 (preventive control) revealed that Bn increase by 105% in the former compared to only 13% in later case. Similarly, ALT and AST values went high by 350 and 220%, respectively in group 4 but but these values changed only 41% and 73%, respectively in preventive control. Urea, Alb and TP levels decreased by 55, 40 and 29%, respectively in group 4 compared to 23.7, 20.7 and 24.2%, respectively in preventive group. These results suggests that the extract dose before the admininsteration of xenobiotic (CCL_4) had helped preventing the damage to liver cells. Continued dose for further three days brought the levels of health markers to almost normal (Table 35). *Euphorbia prostrata* contained 0.7 ± 0.02 mg/gm flavonoids and same quantity of alkaloids. Studies have shown that flavonoids are comparably effective to silymarin in protecting liver health while alkaloid also protects liver from being injured (Palanisamy *et al*., 2007; Yasmin *et al*., 2011).

The overall results showed that biochemical markers of liver health did not change in normal rabbits (Groups 1, 2 and 3), either with standard drug or with plant extract. Treatment of group 4, 5 and 6 with both standard drug and plant extract improved the levels of all biochemical markers. This shows the therapeutic affectivity of plant extract in liver problems. Study of protective group revealed the importance of the plant in prophylactic use. The possible mechanism of liver protection may be the prevention of lipid peroxidation, enhancing protein synthesis, stabilizing cellular membranes and inhibiting cytochrome p-450 (Rao *et al*., 2005).

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Table 36 is set with the changes in values of liver health indicators after treatment with CCl_4. For evaluating the effects of plant extract in healthy rabbits and compare it with positive and negative control, the results are shown in Table 37. To check the therapeutic effect of plant extracts, the results are arranged in Table 38. Table 39 shows the data for preventive control group of rabbits.

Comparison of normal rabbits: In the present study, group 1 was negative control. Like *E. granulata*, in this group also the biochemical markers had not changed. Group 2 was treated with silymarin and group 3 with plant extract. Treatment with silymarin had slightly increased urea level from 24 (± 3.536) to 27 (± 3.391) and AST decreased from 44 (± 3.808) to 41(± 4.301). The rest of the markers remained unchanged during 3 days of experiment. Treatment of group 3 with extract from *E. prostrata* resulted in a slightly decreased (5.2%) in ALT from 58 (± 4.123) to 55 (± 4.301) and AST (8.2%) from 61 (± 6.042) to 56 (± 6.595). Decrease in ALT and AST levels may be due to the repairing or regenerating effect of extract on hepatocytes. The decreased in values of these important enzymes with extract indicate the liver protective effect. In previous study of *E. granulata*, treatment of group 3 with extract had produced no change in the overall biochemical profile. This reveals that *E. prostrata* is more protective than *E. granulata*.

Comparison of CCl_4 treated rabbits: Due to unusual results the group 4 rabbits needed more critical analysis compared to group 5 or 6. The reason for unexpected results in this group might be that the process of hepatic cells necrosis (due to CCl_4) still continued during our analysis. In previous study of *E. granulata*, it was observed that in group 4 the levels of liver health markers continue to deranged up to 3rd day of study. Surprisingly in present study some of the liver health markers showed reversed trend i.e. some keep on improving towards the normal while the other continue to derange. The results show (Table 38) that in group 4 the level of Bn slightly increased by 5% from 9.9 (± 2.118) to 10.4 (± 2.44) while TP decreased by 7.5% from 40 (± 3.542) to 37 (± 3.349). Both these trends indicated problems with liver. It was further observed that urea levels enhanced slightly by 12.3% from 7.3 (± 1.992) to 8.2 (± 1.456), ALT decreased by 13.6%

from 220 (± 20.61) to 190 (± 12.67) and AST decreased by 11.5% from 130 (± 9.49) to 115 (± 9.25). These trends indicate improvement in liver. The possible explanation for decreased ALT and AST levels is that on treatment with CCl_4 hepatocytes were affected but the lyses gradually reduced until all affected cells lysed and no more release of the enzymes. Furthermore the existing hepatocytes could not manage to synthesize the normal level of TP and hence its level continues to decrease. The increased level of Bn indicates the possible problem with bile duct induced with CCl_4. Slight increase of urea indicate the natural regeneration process of hepatic cells or increased activity of existing hepatocytes.

To check the liver toxicity or protective effect of synthetic drugs and natural extract, CCl_4 induced liver necrosis is the best model. CCl_4 produced toxicity in liver by generating trichloromethyl (CCl_3) radical. Trichloromethyl radical stimulates lipid peroxidation by attack membrane phospholipids that lead to cell lyses (Rao *et al*., 2005). Therefore, the CCl_4 treated rabbits model used for *E. granulata* was also tested for *E. prostrata*. Treatment of group 5 with silymarin brought increase in urea, TP and Alb but decreased the AST, ALT and Bn levels. These results are suggestive of improved lever health. Treatment with plant extract needs critical comparison. The present results show that Bn decrease slightly by 7% from 8.6 (± 1.334) to 8(± 1.238) with plant extract compared to 11.3% from 9.7 (± 2.668) to 8.6 (± 1.908) with silymarin. With plant extract ALT decrease by 18.3% from 280 (± 6.671) to 247 (± 6.124) while this decrease was 8.6% from 268 (±9.192) to 245 (± 8.337). Similarly, AST also decreased by 6.3% from 142 (± 7.91) to 133 (± 8.75) with extract and 9.6% from 125 (± 14.58) to 113 (± 13.04) with silymarin. Furthermore the urea level rose by 40% from 10 (± 2.178) to 14(± 1.447) in test group compared to 41.8% from 5.5 ± 1.942 to 7.8 ± 2.123 with standard drug. Both albumin and TP levels did not change with extract.

The present results when compared with the results of *E. granulata*, it was noticed that the levels of albumin and TP had not improved with *E. granulata* extracts but significantly improved with *E. prostrata*. Similarly, urea level increased by only 8.5% with *E. granulata* while it increased by 40% in the present study. This indicates that *E.*

prostrata is more effective in protecting liver than *E. granulata*. Changes in other markers indicate almost identical improvement.

Comparison of preventive groups: The two plants effectively prevented the damaging effects of CCl_4. The three days treatment with *E. prostrata* extract further reveals improvement in liver health (Table 39). Here on day 3 the levels of Bn decreased from 4 (± 1.079) to 3.8 (± 1.056), ALT from 75 (± 4.743) to 70 (± 5.196) and AST from 90 (± 4.123) to 81 (± 4.062).

The overall results reveal that both *E. granulata* and *E. prostrata* are good candidates for protecting the hepatic cells from xenobiotic induce damage while *E. prostrata* is more effective in normalized the functions of affected hepatic cells.

8. **Effect on bone health markers**

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The bone health was monitored from 3 biochemical markers i.e. alkaline phosphatase (ALP), serum calcium and phosphorus. Effects of parathyroid hormones (PTH), dexamethasone and plant extract were monitored on bone health markers for 20 days. The impacts of PTH, plant extract and corticosteroid on bone health markers are summarized in Table 40. The study is focused on investigating the role of *E. granulata* in homeostasis of ALP, calcium and phosphorus.

Comparison of three normal groups: Groups 1, 2 and 3 comprised of normal healthy rabbits with normal physiological levels. Of the three groups, groups 1 was treated with empty capsule shell throughout the experimental period. Levels of ALP, Ca and P were monitored on every 5th day for 20 days. There were no changes in biochemical markers. This indicates that lab environments had no impact on the normal physiology of the rabbits and the results were likely to be reliable. Administration of PTH to group 2 and plant extract to group 3 rabbits produced clear changes in bone related biochemical parameters. The levels of ALP increase significantly ($p<0.05$) with PTH from 32 (± 9.176) to 36 IU (± 8.614) on day 10 of treatment. This indicates that PTH activated the osteoblasts to mineralize the bone. The increase in ALP level was detected at 10^{th} day of the experiment and was maintained with negligible variation till the

experimental period. A similar increase in ALP levels (11.1%) from 36 (± 8.319) to 40 (± 8.258) was also observed with plant extract. Osteoblasts are uni-nucleated cells responsible for construction of bones while osteoclasts are multinucleated cells responsible for destruction or resorption of bone (Aleksyniene *et al*., 2006). As bone making and resorption is a dynamic process and continuously occurs hence both the cells are present all the times to perform their duty. In childhood osteoblasts are more dominant cells while in old age osteoclast overwhelms. The beginning of osteoporosis occurs by attachment of osteoclast to osteon (fundamental unit of compact bone). Osteoclasts releases enzymes collagenase to mobilize small protein and minerals into the extracellular fluid which are the marker of osteoporesis. Osteoclasts then tunnels into inside of mineralized bone for further destruction (Yuk *et al*., 2002). Vitamin D, parthyroid hormones and steroidal hormones are the factors influencing the activities of these cells positively or negatively (Al-Attar. 2010; Basile *et al*., 2006). The increased ALP activity is an indication of increased osteoblasts activity and increased bone mineralization (Li *et al*., 2009). The levels of calcium were augmented by 18.9% from 7.4 (± 0.976) to 8.8 mg/dl (± 1.194) with PTH and by 9.6% from 5.2 (± 0.618) to 5.7 mg/dl (± 0.532). With PTH the serum P levels increased by 22.5% from 4 (± 0.397) to 4.9 mmol/L (± 0.365) on 5^{th} day and was maintained till 20^{th} day. Similarly with extract the P levels rose by 13.1% from 3.8 (0.303) to 4.3 (± 0.552) mmol/L on 5^{th} day and this level continued till 20^{th} day. Although statistically not significant ($p>0.05$), but the levels of both Ca and P increased clearly with plant extract. This none significant increase in Ca and P is also important because this increase occurred with crude extract and there is the possibility of more effective pure compound in plant. The overall effect on Ca and P seems that the extract may have increased intestinal absorption, decrease nephritic secretion or mobilization from bones which shows the identical role with PTH.

Comparison of three treated groups: Group 4 received dexamethasone on daily basis without receiving any treatment. This group needs critical discussions due to the changes that occurred. High levels of corticosteroids directly results in changes of osteoblasts and osteoclasts activity (Li *et al*., 2009). Table 40 shows a clear and abrupt ($p>0.05$) fall of ALP from 32 (± 6.042) to 29(± 5.831) on 5^{th} day and then base line level

32 (± 5.718) was resumed on 20th day. In this group Ca levels increased significantly ($p<0.05$) from 6.1 (± 1.167) to 6.4 (± 1.083) while P levels rose from 4.2 (± 0.402) to 4.4 (±0.593) on 10th day. These levels then normalized to 5.7 (± 0.919) and 3.9 (± 0.259), respectively. The end values were close to the base line levels. The sharp increase in calcium and phosphorus levels with corticosteroids indicates increased in number and activity of osteoclast which enhances bone resorption. Studies shows that corticoids influence body physiology during the initial period of therapy and then normalizes if used chronically (Yasear & Hamouda, 2009). The above acute drop and then normalization of ALP might have occurred due to this property of steroidal therapy. The exact mechanism by which bone resorption occurs with corticosteroids is not known but increase in osteoclasts activity could be one of the reasons. The decrease in Ca and P levels may be due to decrease absorption or increase secretion or may be due to cellular influx. Comparing the impact of administering the PTH in group 5 and plant extract in group 6 showed that PHT enhanced the level of ALP by 16.7% from 36 (± 6.38) to 42 IU/L (± 6.686) at 5th day and then maintained at this level. However with plant extract the ALP levels ALP rose by 13.6% from 22 (± 4.817) to 25 IU/L (± 5.357). Increase of ALP is the direct effect of PTH on osteoblast activity to mineralize the bone structure. This indicate that PTH overwhelm the bone desorption and osteoclast stimulating activity of steroids (Aleksyniene *et al*., 2006). Calcium levels also improved both with PTH and plant extract. The Ca levels sharply went high from 5.7 (± 1.171) to 6.4 (± 1.297) till 10th day in the PTH treated rabbits and thereafter maintained this level till 20th days. In case of plant extract the Ca level increased from 7.2 (± 0.78) to 7.6 (± 0.54) on day 10th and then dropped to a value (7.4 ± 0.754) very close to the base line level. The results revealed that this group shows improvement in levels of all the three variables (ALP, Ca and P). Although, smaller than the effect of PTH on the three variables (ALP, Ca and P), yet the results showed that the plant extract also exerted identical effects to PTH on these biochemical markers. Increase activity of ALP with plant extract may be the PTH like activity of plant extract. The increase calcium and phosphorus levels may also be due to the increased absorption from food. Another possibility may be the presence of vitamin D

in the plant extract (Ambroszkiewicz *et al*., 2007). These effects suggest the usefulness of therapeutic use of this plant in osteoporosis.

Comparison of three treated with three healthy groups: The three non-treated groups differ from treated on the grounds that in first case the drugs and extract exerts its effect in rabbits of normal physiology. In the case of treated groups the drug and extract produces its effect in a steroid treated disturbed physiology. Groups 1 and 4 (both negative control) shows slight difference when compared. Entire variables (ALP, Ca and P) sustained throughout the experimental period in group 1. This occurred on account that there were no interfering factors in body physiology. In case of group 4, dexamethasone negatively influenced ALP, while Ca and P increased in the beginning but then returned to normal. In group 2, PTH exerted its positive effects on bone without the negative impacts of corticosteroids. In this case the markers indicated improvement in bone health. In group 5, PTH positively influenced bone health in presence of the negative impact of dexamethasone. The results overall pointed out that the levels of ALP, Ca and P improved but not as significantly as in group 2. Finally in group 3 (tested group) the extract effect on ALP, Ca and P was monitored. In this set of rabbits all the bone health markers showed improvement both in normal and corticosteroid treated rabbits i.e. ALP, Ca and P increased. In group 6 (tested group) the improvement of bone health indicators (ALP, Ca and P) with plant extract was monitored in presence of negative influence of corticosteroids. In both normal and treated groups the indicators showed significant improvement of bone health. Before 2002, in postmenopausal women hormone replacement therapy or HRT was used to treat osteoporosis. Due to reports of breast cancer, cardiac problems, blood clots and strokes American National Institute of Health stopped use of HRT (Burali *et al*., 2010).

Summing the results shows that the plant extracts showed biochemical results identical with PTH. This might be hormones like effect or might have helped increasing the secretion of hormones responsible for bone health' i.e. PTH and calcitonin (Yakubu, *et al*., 2006). The effects might also be due to the excess presence of vitamin D in plant extract (Ambroszkiewicz *et al*., 2007). The plant needs further investigation of its influence on secretion of bone related hormones (PTH and calcitonin). Microtomography

is needed to check the impact of plant on bone mass density (BMD) and bone mass volume (BMC).

***Euphorbia prostrata* Aiton**

This plant was also screened for bone health impact like *E. granulata*. The results are shown in Table 41. The results of both *E. granulata* and *E. prostrata* have been compared.

Comparison of three normal groups: In group 1 (normal control), the three markers did not changed during the experiment. Rabbits in this group were not treated and hence this suggested the correctness of our experimental procedure. Group 2 were healthy rabbits treated with PTH hormones. In this group ALP rose significantly by 25% ($p<0.001$) from 36 (± 1.304) to 45 IU/L (± 1.871), Ca by 17% from 4.7 (± 0.415) to 5.5 mg/dl (± 0.251). Phosphorus level also enhanced by 15.8%, from 3.8 (± 0.141) to 4.4 mmol/L (± 0.255) in group 2. Levels of blood calcium and vitamin D are strong factors for enhancing bone mineralization and preventing osteoporosis. Presence of calcium in diet and its absorption is also crucial for good health of bone (Ambroszkiewicz *et al*., 2007). The results indicate the influence of PHT on increased number and activity of osteoblasts and hence increased mineralization. Group 1 and 2 were standard groups with standard treatments therefore both in previous (*E. granulata*) and in present (*E. prostrata*) study identical results were produced. Group 3 was treated with *E. prostrata* extract. In this group, ALP increased significantly ($p<0.001$) by 20% from 20 (± 1.483) to 24 IU/L (± 2.49). Plants are not only source of calcium and phosphorus but also contain factors that influence its absorption and bone mineralization. Blood calcium and phosphorus level usually reflect the bone and calcium regulatory hormones related diseases (Al-Attar, 2010; Li *et al*., 2009). This effect of the plant extract indicated the direct stimulant effect of extract on the osteoblast activity or may be a direct effect of influencing the secretion or inhibition of PHT or calcitnin (Basile *et al*., 2006). In this group Ca and P none significantly increase from 6.2 to 6.5 (4.8%) and 5.1 to 5.5 (7.8) respectively. Enhanced levels may be due to increased mobilization from bone but the increased ALP indicates that these levels are not due to mobilization but increased absorption (Al-Attar, 2010; Li

et al., 2009). The results for *E. granulata* and *E. prostrata* are identical which further suggest the similarity in biochemical behaviors of these two plants.

Comparison of three treated groups: In group 4, the levels of ALP dropped initially from 23 (± 1.0) to 21.2 (± 0.837) on 5th day of experiment. This group latter on resumed the base line level on 10th day and keep it onward. This suggests the osteoblast suppressing effect of corticosteroids (Li *et al*., 2009). Corticoids act further to increase the Ca level significantly ($p<0.05$) from 7.7 (± 0.597) to 8.22 (± 1.158) and P levels from 3.6 (± 0.303) to 4 (± 0.187). These levels then normalized to nearly base line levels at 20th day of experiment. Initial acute bone loss and the slow normalization to a level below base line is a feature of glucocorticoid treatment (Aleksyniene *et al*., 2006). In group 5, the bones were under the negative influence of corticosteroids and the positive impact of PTH. The results show that unlike group 4, the levels of all bone health markers increased significantly ($p<0.05$). ALP activity increased by 20% from 30 (± 1.871) to 36 (± 1.304), Ca by 13.9% from 7.2 (± 1.665) to 8.2 (± 1.506) and P by 22.5% from 4 (± 0.351) to 4.9 (± 0.458). Table 41 further reveal that the values increased slowly till 5 days and then attained a stable level which continued for 20 days. This suggests that initially the effects of corticosteroid balanced the activity of PTH but latter the positive role of PTH overwhelm the corticosteroids. This effect in group 5 differs from group 4 in the levels of Ca and P. Group 6 was treated with 200 mg test extracts. The effects of *E. prostrata* were surprisingly similar to the combined effect of corticosteroid and PTH treated group. In this group, ALP activity increased slowly and significantly ($p<0.01$) by 19.2% from 26 (± 1.304) to 31 (± 1.225), Ca by 6.1% from 8.2 (± 1.319) to 8.7 (± 1.268) and P by 10% from 4 (± 0.416) to 4.4 (± 0.472). Increased activity of ALP along with Ca and P levels suggests that the extracts not only stimulated mineralization of bones through activation of osteoblasts but also enhanced the absorption of Ca and P. The extracts of *E. granulata* also produced similar effects.

It is reported that certain plant compounds like flavonoids, lignans, and coumestans have structural similarity to natural or synthetic oestrogens. These compounds have the ability to mimic or modulate the activity of natural endogenous oestrogens by binding to their receptors (Li *et al*., 2009). Flavonoids were found in *E.*

granulata and *E. prostrata.* The antiosteoporotic potential of the two plants suggests that both contain good amount of these active principals.

9. Effects on electrolyte levels

***Euphorbia granulata* Forssk**

The results for the impact of furosemide as positive control and that of different test doses of *E. granulata* extract on rabbits are given in Table 42.

Excessive intake of salt can leads to many complications (Ikpi *et al*., 2009). Kidneys have the key role in maintaining and regulating water, electrolyte and acid base balance (Ezekwesili *et al*., 2008; Ofem *et al*., 2010). Therefore, a decline in kidney functions and consumption of protein rich diet may lead to metabolic acidosis and accumulation of electrolytes in blood (Eberechukwu *et al*., 2007). Chronic diarrhea causes negative electrolytes balance especially hyponatremia (Oluwole, 2001). The present results reveal that group 1 maintained the normal level of sodium, potassium and chloride ions throughout experimental period. No change in electrolytes level of group 1 rabbits was clear from the fact that they were not induced with any stimulant of inhibitor for doing so. Slight increase of potassium from 4.3 (± 0.577) to 4.44 (± 0.23) in negative control group was noted that might be considered as temporary and dietary effect. Diuretics like frusemide, mannitol, ethacrinic and thiazides helps in relieving from fluid overload (Shivalinge *et al*., 2009). In present study, group 2 had significant ($p<0.01$) decrease in Na^+ level, from 145 (± 5.385) to 124 (± 2.55) with frusemide. Plants constituents either directly influences the function of kidneys as diuretics or they stimulate or inhibit the secretion of body hormones. For example, they influence aldosterone and anti-diuretic hormones involved in secretion and retention of electrolytes (Ofem *et al*., 2010). No change was observed in Na^+ levels either with 50, 100, 150 or 200 mg extracts treatment. This suggests that extract had no role in the blood Na^+ levels. Furosemide reduced the Cl^- levels from 105 (± 4.472) to 96 (± 4.123) mmol/l in group 2 rabbits while plant extract was found ineffective in reducing the Cl^- level in any of the treated groups. This is indicative of lack of any role of plant either in secretion or absorption of Cl^-. In group 2 the values of potassium rose significantly ($p<0.05$) from 4.4

(± 0.212) to 5.5 (± 0.412) with standard drug but again no detectable change was noted with plant extract. Synthetic diuretic induces sodium chloride (NaCl) secretion but retain potassium (K^+) because excretion of each chloride ions follows a sodium ion (Ofem *et al*., 2010). That is why in group 2 the level of K^+ increased from base line level against the sodium ion.

Although no significant change in electrolytes levels was observed with plant extract treatment yet slight variation was found at 150 mg dose in group 5 rabbits. In this group Na changed by 2.1% from 143 (± 3.536) to 140 (± 4.123), K by 5.3% from 5.7 (± 0.158) to 5.4 (± 0.474) and Cl by 3.8% from 106 (± 4.123) to 110 (±4.472). Out of the four tested groups it was the only group with such a clear change in electrolytes values. Negligible variations were also noticed for group 4. Group 6 received larger dose (200 mg/kg) than group 5 but even then the electrolyte levels did not changed from base line. For diuretic effect "if any" in plant, 150 mg seems to be the effective dose.

The overall results suggest that there was no clear cut therapeutic effect due to these plants at the tested doses and time interval. The effect of 150 mg/kg dose needs further trails, as to whether the dose is really an effective dose for diuresis or it was an accidental variation in rabbit's electrolytes levels. Similarly the study also need to be extended weather the extract doses for long time treatment have any effect on the electrolytes levels.

***Euphorbia prostrata* Aiton**

The results of electrolyte levels in rabbits before and after receiving standard drug are presented in Table 43. In negative control group, the levels of sodium at zero time were 133 (± 2.236) which slightly dropped to 130 (± 4.123). K level remain constant while Cl showed a small drop from 113 (± 2.55) to 111 (± 5.099). Logically electrolytes levels should not be changes in negative control. The changes in the present results are insignificant and seem the effect of water and food uptake rather than any physiologic effect. In positive control group the values Na^+ dropped significantly ($p<0.05$) by 12% from 135 (± 3.162) to 119 (± 2.236). The levels of electrolytes with 100, 150 and 200 mg extract doses were not affected. Groups 4, 5 and 6 sustained the base line level throughout 5 days of experiment (Table 43). Small variations in Na level in these groups

are comparable to change in negative control. These variations were insignificant that might be the effects of water and food uptake. Only with 50 mg the Na levels slightly dropped from 140 (± 2.915) to 138 (± 2.55). The levels of Cl dropped by 7.7% from 104 (± 5.099) to 96 (± 4.743) with drug but all the doses of extract were ineffective in all groups of rabbits. The K^+ levels rose by 9.8% from 4.1 (± 0.158) to 4.5 (± 0.381) in positive control group but dropped by 9% in test group at high dose of 200 mg. It is well known that synthetic diuretic influences the secretion of NaCl and the cellular uptake of K^+ (Ofem *et al*., 2010). The levels of electrolytes in blood may be increased and decrease with cellular release and uptake or by urinary retention and secretion (Khan *et al*., 2003). No major effect was observed in electrolyte levels with different doses of plant extract except K. Potassium level decrease clearly but insignificantly with 200 mg extract. Although statistically not significant ($p>0.05$) but the decrease was sufficient to create a doubt of effect with plant extract. With 200 mg extracts, Na and Cl levels were not affected therefore it can be said that this dose was effective for potassium only. This may be achieved either by increased secretion or increased cellular uptake.

The overall results showed that both *E. granulata* and *E. prostrata* lack clear activity to influence the electrolytes levels. However, 150 mg dose of *E. granulata* and 200 mg dose of *E. prostrata* had some effect.

Table 20. Effect of ethanolic extracts of *E. granulata* on BGL in groups of rabbits.

Experimental group	BGL before and after treatment with plant extract								
	0 hour	1 /2hr	1 hr	2 hrs	4 hrs	8 hrs	24 hrs	48 hrs	72 hrs
Group 1	96 ± 5.891	95 ± 6.221	95 ± 7.014	96 ± 5.891	95 ± 5.718	95 ± 4.93	96 ± 5.404	96 ± 6.269	95 ± 6.301
Group 2	103 ± 6.671	100 ± 6.458	98 ± 6.269	88 ± 5.07	84 ± 5.263	82 ± 5.03	103 ± 5.788	101 ± 5.45	103 ± 6.87
Group 3	101 ± 5.612	100 ± 5.586	99 ± 5.657	98 ± 6.181	96 ± 6.245	96 ± 5.899	96 ± 6.0	97 ± 5.899	103 ± 6.87
Group 4	222 ± 8.989	222 ± 10.01	222 ± 9.884	222± 9.37	222 ± 9.203	222 ± 9.985	222 ± 8.556	222 ± 8.173	222 ± 8.591
Group 5	222 ± 8.927	220 ± 9.731	212 ± 9.823	205 ± 8.792	198 ± 7.95	194 ± 8.081	218 ± 9.182	219 ± 9.566	224 ± 8.689
Group 6	212 ± 13.864	210 ± 13.11	209 ± 12.896	208 ± 13.964	206 ± 13.68	205 ± 13.58	205 ± 13.372	209 ± 11.41	209 ± 12.3

Values are the mean of five observations with ± SD

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 10 mg / kg glibenclemide)

Group 3 = Normal tested (received 200 mg / kg EG extract)

Group 4 = Streptozotocin treated negative Control (received empty capsule shell)

Group 5 = Streptozotocin treated positive Control (received 10 mg / kg glibenclemide)

Group 6 = Streptozotocin treated tested (received 200 mg / kg EG extract)

Table 21. Effect of ethanolic extracts of *E. prostrata* on BGL in groups of rabbits.

Experimental group	BGL before and after treatment with plant extract								
	0 hour	1 /2hr	1 hr	2 hrs	4 hrs	8 hrs	24 hrs	48 hrs	72 hrs
Group 1	84 ± 6.656	83 ± 6.221	83 ± 5.831	83 ± 7.266	83 ± 6.309	84 ± 6.205	84 ± 7.155	83 ± 6.841	83 ± 6.907
Group 2	90 ± 7.635	86 ± 7.981	80 ± 7.403	76 ± 7.537	71 ± 6.87	68 ± 6.979	90 ± 8.012	90 ± 7.503	90 ± 7.759
Group 3	101 ± 5.612	103 ± 5.263	101 ± 5.523	100 ± 5.339	99 ± 5.586	98 ± 5.586	95 ± 4.336	98 ± 5.357	101 ± 5.933
Group 4	224 ± 8.764	223 ± 8.355	224 ± 8.672	222 ± 8.385	223 ± 8.689	223 ± 8.916	223 ± 8.258	223 ± 9.302	223 ± 8.815
Group 5	223 ± 11.696	219 ± 11.43	210 ± 12.116	203 ± 11.52	198 ± 9.965	193 ± 8.264	222 ± 11.884	221 ± 12.47	222 ± 12.41
Group 6	220 ± 14.481	221 ± 14.87	218 ± 14.81	217 ± 14.74	215 ± 14.4	214 ± 14.29	213 ± 14.4	215 ± 14.4	217 ± 14.41

Values are the mean of five observations with ± SD

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 10 mg / kg glibenclemide)

Group 3 = Normal tested (received 200 mg / kg EP extract)

Group 4 = Streptozotocin treated negative Control (received empty capsule shell)

Group 5 = Streptozotocin treated positive Control (received 10 mg / kg glibenclemide)

Group 6 = Streptozotocin treated tested (received 200 mg / kg EP extract)

Table 22. Lipid profile of rabbits before and 2 days after treatment with Isoprenaline.

Experimental group		Lipid profile (mg/dl) before and after administration of Isoprenaline					
		TC	HDL	LDL	VLDL	TG	FFA
Group 4	Before	45 ± 7.28	29 ± 5.31	27 ± 4.658	21 ± 3.701	47 ± 6.181	57 ± 3.347
	After	81 ± 14.7	36 ± 4.301	36 ± 4.97	32 ± 4.438	74 ± 7.106	70 ± 4.583
Group 5	Before	52 ± 10.01	32 ± 3.271	26 ± 3.937	18 ± 3.082	48 ± 4.604	53 ± 4.062
	After	87 ± 13.55	36 ± 3.347	36 ± 3.701	26 ± 3.701	77 ± 5.339	63 ± 3.24
Group 6	Before	58 ± 9.985	32 ± 3.033	28 ± 4.062	21 ± 4.147	51 ± 5.263	56 ± 4.55
	After	83 ± 9.176	34 ± 3.493	38 ± 3.701	30 ± 3.962	71 ± 6.221	65 ± 6.083
Grp 10	Before	46 ± 7.014	37 ± 4.848	29 ± 3.701	21 ± 3.606	42 ± 4.919	54 ± 3.808
	After	48 ± 6.419	39 ± 4.95	31 ± 3.347	20 ± 4.324	45 ± 4.301	52 ± 3.899

Values are the mean of five replicates with ± SD, TC = total cholesterol, HDL = high density lipids, LDL = low density lipids, VLDL = very low density lipids, TG = triglycerides and FFA = free fatty acids

Table 23. Effect of *Euphorbia granulata* extracts on lipid profile in normal rabbits.

Exp. gp	Lipid profile (mg/dl) before and after administering plant extract						
	Interval	TC	HDL	LDL	VLDL	TG	FFA
Group 1	Day 1	54 ± 5.848	33 ± 5.404	29 ± 5.933	21 ± 5.215	42 ± 6.496	55 ± 7.829
	Day 2	55 ± 6.181	33 ± 5.805	30 ± 5.148	19 ± 4.637	42 ± 7.45	55 ± 8.142
	Day 3	55 ± 6.834	33 ± 4.817	29 ± 6.042	21 ± 4.147	43 ± 8.106	56 ± 7.629
Group 2	Day 1	50 ± 6.017	31 ± 4.062	29 ± 4.528	21 ± 3.768	44 ± 3.674	57 ± 5.07
	Day 2	45 ± 5.718	31 ± 3.674	26 ± 3.962	21 ± 3.564	42 ± 3.493	54 ± 3.962
	Day 3	43 ± 5.339	30 ± 3.962	24 ± 3.317	21 ± 3.808	40 ± 2.608	51 ± 4.278
Group 3	Day 1	59 ± 5.523	29 ± 5.05	35 ± 3.493	21 ± 4.658	44 ± 5.63	51 ± 4.207
	Day 2	54 ± 8.701	29 ± 3.633	33 ± 3.362	19 ± 3.606	43 ± 4.324	52 ± 4.301
	Day 3	52 ± 8.585	29 ± 4.324	32 ± 2.881	18 ± 3.674	44 ± 5.874	52 ± 4.147

Values are the mean of five replicates with ± SD, TC = total cholesterol, HDL = high density lipids, LDL = low density lipids, VLDL = very low density lipids, TG = triglycerides and FFA = free fatty acids

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 3mg/kg resveratrol)

Group 3 = Normal tested (received 200 mg / kg plant extract)

Table 24. Effect of *E. granulata* extracts on lipid profile in isoprenaline treated rabbits.

Exp. gp	Lipid profile (mg/dl) before and after administering plant extract						
	Interval	TC	HDL	LDL	VLDL	TG	FFA
Group 4	Day 1	81 ± 15.94	36 ± 4.301	36 ± 4.97	32 ± 4.438	74 ± 7.106	70 ± 4.583
	Day 2	82 ± 14.99	33 ± 4.604	34 ± 5.933	34 ± 4.743	73 ± 6.364	71 ± 4.301
	Day 3	83 ± 14.79	35 ± 3.742	34 ± 5.244	34 ± 4.817	73 ± 5.718	72 ± 4.604
Group 5	Day 1	87 ± 13.73	38 ± 4.382	36 ± 3.701	26 ± 3.701	77 ± 5.339	63 ± 3.24
	Day 2	82 ± 13.01	38 ± 4.528	33 ± 3.847	23 ± 3.391	77 ± 4.97	60 ± 3.606
	Day 3	80 ± 13.44	36 ± 4.266	32 ± 4.336	23 ± 3.317	78 ± 5.119	57 ± 3.209
Group 6	Day 1	83 ± 9.487	34 ± 3.493	38 ± 3.701	30 ± 3.962	71 ± 6.221	65 ± 6.693
	Day 2	77 ± 9.284	34 ± 3.962	34 ± 3.536	25 ± 4.062	69 ± 4.382	66 ± 5.933
	Day 3	77 ± 9.45	34 ± 3.937	35 ± 3.899	25 ± 3.701	69 ± 4.658	66 ± 6.017

Values are the mean of five replicates with ± SD, TC = total cholesterol, HDL = high density lipids, LDL = low density lipids, VLDL = very low density lipids, TG = triglycerides and FFA = free fatty acids

Group 4 = Isoprenaline treated negative Control (received empty capsule shell)

Group 5 = Isoprenaline treated positive Control (received resveratrol)

Group 6 = Isoprenaline treated tested (received 200 mg / kg plant extract)

Table 25. Effect of *Euphorbia granulata* extracts on lipid profile in diabetic rabbits.

Exp. gp	Lipid profile (mg/dl) before and after administering plant extract						
	Interval	TC	HDL	LDL	VLDL	TG	FFA
Group 7	Day 1	82 ± 6.496	41 ± 3.674	33 ± 4.062	21 ± 3.082	70 ± 3.701	69 ± 2.588
	Day 2	80 ± 6.834	40 ± 3.701	34 ± 4.147	20 ± 3.742	70 ± 3.24	65 ± 3.162
	Day 3	82 ± 6.934	40 ± 3.768	34 ± 3.742	21 ± 3.768	69 ± 2.775	65 ± 3.834
Group 8	Day 1	69 ± 7.396	40 ± 3.808	36 ± 4.301	22 ± 4.97	71 ± 3.082	70 ± 3.347
	Day 2	65 ± 6.76	40 ± 3.362	33 ± 3.633	21 ± 4.266	72 ± 2.683	66 ± 3.647
	Day 3	61 ± 5.63	41 ± 3.493	31 ± 3.0	19 ± 4.506	69 ± 3.564	65 ± 2.702
Group 9	Day 1	75 ± 3.271	44 ± 3.162	42 ± 2.55	23 ± 3.962	74 ± 2.387	69 ± 2.55
	Day 2	70 ± 3.701	44 ± 3.271	40 ± 1.304	20 ± 4.438	74 ± 1.581	69 ± 2.588
	Day 3	70 ± 3.962	45 ± 2.588	39 ± 1.581	19 ± 3.674	73 ± 2.387	72 ± 2.28

Values are the mean of five replicates with ± SD

TC = total cholesterol, HDL = high density lipids, LDL = low density lipids, VLDL = very low density lipids, TG = triglycerides and FFA = free fatty acids

Values are the mean of five replicates with ± SD

Group 7 = Diabetic negative control (received empty capsule shell)

Group 8 = Diabetic positive control (received resveratrol)

Group 9 = Diabetic tested (received 200 mg / kg plant extract)

Table 26. Effect of *Euphorbia granulata* extracts on lipid profile in diabetic rabbits.

Exp. gp	Lipid profile (mg / dl) before and after administering plant extract						
	Interval	TC	HDL	LDL	VLDL	TG	FFA
Group 10	Day 1	48 ± 6.419	39 ± 4.95	31 ± 3.347	20 ± 4.324	45 ± 4.301	52 ± 3.899
	Day 2	47 ± 7.19	40 ± 4.301	32 ± 3.033	18 ± 4.324	43 ± 3.114	52 ± 4.382
	Day 3	47 ± 6.723	40 ± 4.604	31 ± 2.588	18 ± 3.674	44 ± 3.768	51 ± 4.324

Values are the mean of five replicates with ± SD, TC = total cholesterol, HDL = high density lipids, LDL = low density lipids, VLDL = very low density lipids, TG = triglycerides and FFA = free fatty acids

Group 10 = Preventive control (received 200 mg / kg plant extract along with isoprenaline)

Table 27. Comparative lipid profile of rabbits before and after treatment with Isoprenaline.

Experimental group		Lipid profile (mg/dl) before and after administration of Isoprenaline					
		TC	HDL	LDL	VLDL	TG	FFA
Group 4	Before	35 ± 4.919	24 ± 3.937	23 ± 2.95	16 ± 3.033	41 ± 3.768	60 ± 6.496
	After	61 ± 5.718	31 ± 3.937	33 ± 5.07	28 ± 3.701	67 ± 4.583	77 ± 6.124
Group 5	Before	42 ± 5.541	28 ± 6.042	25 ± 4.95	16 ± 4.97	41 ± 5.119	59 ± 5.244
	After	67 ± 8.438	33 ± 6.099	32 ± 5.263	22 ± 4.743	68 ± 6.648	69 ± 5.148
Group 6	Before	48 ± 3.701	28 ± 4.438	24 ± 5.45	18 ± 4.062	43 ± 4.817	59 ± 4.604
	After	63 ± 4.95	31 ± 4.416	32 ± 5.523	25 ± 3.033	64 ± 4.97	69 ± 3.768
Grp10	Before	36 ± 2.28	34 ± 5.148	25 ± 4.817	18 ± 4.743	38 ± 8.012	58 ± 7.53
	After	49 ± 3.536	36 ± 3.606	25 ± 4.472	16 ± 4.438	39 ± 7.649	57 ± 5.916

Values are the mean of five replicates with ± SD

TC = total cholesterol, HDL = high density lipids, LDL = low density lipids, VLDL = very low density lipids, TG = triglycerides and FFA = free fatty acids

Table 28. Effect of *Euphorbia prostrata* extracts on lipid profile in normal rabbits.

Exp. gp	Lipid profile (mg/dl) before and after administering plant extract						
	Interval	TC	HDL	LDL	VLDL	TG	FFA
Group 1	Day 1	46 ± 3.028	29 ± 2.915	24 ± 2.915	17 ± 3.899	40 ± 4.324	60 ± 5.31
	Day 2	45± 4.062	29 ± 2.864	22 ± 2.864	17 ± 3.421	41 ± 2.55	59 ± 5.701
	Day 3	46 ± 3.493	28 ± 2.55	24 ± 4.438	16 ± 2.739	39 ± 3.033	60 ± 6.042
Group 2	Day 1	41 ±3.564	29 ± 2.236	26 ± 6.181	17 ± 4.583	39 ± 3.082	61 ± 3.493
	Day 2	37 ± 3.606	30 ± 1.414	23 ± 5.762	15 ± 3.421	38 ± 3.391	57 ± 3.114
	Day 3	35 ± 2.915	29 ± 1.414	22 ± 5.32	14 ± 2.702	38 ± 3.082	55 ± 2.881
Group 3	Day 1	48 ± 4.817	29 ± 6.042	25 ± 4.55	18 ± 4.301	39 ± 3.937	54 ± 5.612
	Day 2	45 ± 3.674	30 ± 5.07	24 ± 4.712	17 ± 4.025	38 ± 3.114	56 ± 5.916
	Day 3	43 ± 3.674	31 ± 4.817	25 ± 4.712	14 ± 2.95	39 ± 3.271	55 ± 5.933

TC = total cholesterol, HDL = high density lipids, LDL = low density lipids, VLDL = very low density lipids, TG = triglycerides and FFA = free fatty acids

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received resveratrol)

Group 3 = Normal tested (received 200 mg / kg plant extract)

Table 29. Effect of *E. prostrata* extracts on lipid profile in isoprenaline treated rabbits.

Exp. gp	Lipid profile (mg/dl) before and after administering plant extract						
	Interval	TC	HDL	LDL	VLDL	TG	FFA
Group 4	Day 1	60 ± 7.176	31 ± 3.937	31 ± 4.301	27 ± 4.382	68 ± 5.612	75 ± 4.025
	Day 2	61 ± 5.701	31 ± 4.438	30 ± 4.919	27 ± 4.301	68 ± 4.817	76 ± 4.743
	Day 3	61 ± 6.124	30 ± 4.301	31 ± 4.207	28 ± 5.07	68 ± 5.541	76 ± 4.382
Group 5	Day 1	66 ± 9.354	33 ± 6.099	31 ± 4.604	22 ± 4.743	71 ± 6.042	66 ± 4.025
	Day 2	62 ± 8.877	34 ± 4.97	28 ± 4.669	20 ± 4.336	72 ± 5.495	63 ± 3.082
	Day 3	61 ± 8.562	34 ± 4.712	27 ± 4.712	19 ± 3.578	71 ± 5.357	61 ± 3.701
Group 6	Day 1	64 ± 4.95	30 ± 5.244	33 ± 5.675	26 ± 3.082	65 ± 5.119	69 ± 3.768
	Day 2	58 ± 4.658	29 ± 4.97	30 ± 4.604	20 ± 3.347	64 ± 5.718	68 ± 3.082
	Day 3	57 ± 5.148	30 ± 5.263	30 ± 4.848	21 ± 3.347	65 ± 5.612	68 ± 3.674

Values are the mean of five replicates with ± SD, TC = total cholesterol, HDL = high density lipids, LDL = low density lipids, VLDL = very low density lipids, TG = triglycerides and FFA = free fatty acids

Group 4 = Isoprenaline treated negative Control (received empty capsule shell)

Group 5 = Isoprenaline treated positive Control (received resveratrol)

Group 6 = Isoprenaline treated tested (received 200 mg / kg plant extract)

Table 30. Effect of *Euphorbia prostrata* extracts on lipid profile in diabetic rabbits.

Exp. gp	Lipid profile (mg/dl) before and after administering plant extract						
	Interval	TC	HDL	LDL	VLDL	TG	FFA
Group 7	Day 1	73 ± 7.014	34 ± 6.181	28 ± 4.95	17 ± 6.671	65 ± 8.031	73 ± 7.778
	Day 2	71 ± 6.686	35 ± 5.612	28 ± 4.438	17 ± 5.848	66 ± 8.86	71 ± 7.085
	Day 3	71 ± 7.28	35 ± 5.975	28 ± 4.147	17 ± 6.403	65 ± 8.307	72 ± 6.595
Group 8	Day 1	70 ± 6.979	38 ± 5.45	31 ± 5.385	17 ± 2.168	65 ± 3.701	76 ± 4.743
	Day 2	66 ± 6.542	39 ± 6.058	28 ± 4.393	16 ± 1.924	66 ± 4.604	72 ± 4.266
	Day 3	65 ± 6.348	39 ± 5.541	26 ± 3.391	14 ± 1.517	65 ± 4.604	71 ± 5.916
Group 9	Day 1	72 ± 5.891	40 ± 4.438	37 ± 3.768	19 ± 4.637	68 ± 3.493	77 ± 4.062
	Day 2	72 ± 6.124	39 ± 4.95	37 ± 3.564	16 ± 3.834	67 ± 4.324	75 ± 3.962
	Day 3	73 ± 5.805	40 ± 4.868	37 ± 3.674	16 ± 3.536	67 ± 3.742	77 ± 4.95

Values are the mean of five replicates with ± SD, TC = total cholesterol, HDL = high density lipids, LDL = low density lipids, VLDL = very low density lipids, TG = triglycerides and FFA = free fatty acids

Group 7 = Diabetic negative control (received empty capsule shell)

Group 8 = Diabetic positive control (received resveratrol)

Group 9 = Diabetic tested (received 200 mg/kg plant extract)

Table 31. Effect of *Euphorbia prostrata* extracts on lipid profile in diabetic rabbits.

Exp. gp	Lipid profile (mg/dl) before and after administering plant extract						
	Interval	TC	HDL	LDL	VLDL	TG	FFA
Group 10	Day 1	49 ± 3.536	36 ± 3.606	25 ± 4.472	16 ± 4.438	39 ± 7.649	57 ± 5.916
	Day 2	47 ± 3.962	36 ± 2.828	25 ± 3.701	14 ± 3.768	39 ± 6.964	56 ± 5.431
	Day 3	48 ± 3.564	37 ± 3.606	25 ± 3.962	14 ± 4.025	40 ± 7.823	56 ± 5.805

Values are the mean of five replicates with ± SD, TC = total cholesterol, HDL = high density lipids, LDL = low density lipids, VLDL = very low density lipids, TG = triglycerides and FFA = free fatty acids

Group 10 = Preventive control (received 200 mg/kg plant extract along with isoprenaline)

Table 32. Levels of liver health markers before and after administration of CCl_4.

Exp. groups		Liver health markers					
		Bn (µmol/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)	Alb (g/dl)	TP (g/dl)
Group 4	Before	3.41 ± 0.607	15 ± 3.808	60 ± 3.536	36 ± 3.536	29.1 ± 3.09	59.9 ± 4.5
	After	7 ± 0797	6.7 ± 0.64	270 ± 11.4	115 ± 11.7	17.5 ± 3.84	42.5 ± 5.85
	% Eff.	105.3	55.3	350	220	40	29
Group 5	Before	4.36 ± 0.746	18 ± 2.236	58 ± 4.123	61 ± 2.828	32.4 ± 1.81	66.1 ± 4.5
	After	8.2 ± 0.495	6.1 ± 0.886	245 ± 13.29	130 ± 16.08	17 ± 3.386	38 ± 2.739
	% Eff.	88	66	322	113	47.5	42.5
Group 6	Before	3.47 ± 0.665	19 ± 2.55	64 ± 4.743	48 ± 5.385	31.9 ± 4.2	67.5 ± 3.64
	After	7.2 ± 0.914	7.2 ± 0.914	248 ± 13.32	115 ± 11.22	14 ± 3.26	44 ± 6.285
	% Eff.	107	62	288	140	56	35
Group 7	Before	5.4 ± 1.14	16 ± 3.082	71 ± 2.55	45 ± 5.099	30.9 ± 4.21	54.1 ± 2.93
	After	6.1 ± 0.893	12.2 ± 3.99	100 ± 10.6	78 ± 6.519	24.5 ± 2.72	41 ± 4.848
	% Eff.	13	23.7	41	73.3	20.7	24.2

Values are the mean of five replicates with ± SD, Bn = bilirubin, Alb = albumin, TP = total protein and %Eff = percent effect.

Table 33. Effect of *E. granulata* extracts on liver health markers in healthy rabbits.

Exp. gp	Levels of liver health markers during experiment						
	Interval	Bn (µmol/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)	Alb (g/dl)	TP (g/dl)
Group 1	Day 1	4.4 ± 0.474	21 ± 3.536	57 ± 6.442	57 ± 3.674	32.2 ± 3.04	52.8 ± 5.02
	Day 2	4.2 ± 0.543	21 ± 3.082	55 ± 7.05	57 ± 4.359	33 ± 2.915	50 ± 3.937
	Day 3	4.3 ± 0.442	21 ± 3.674	55 ± 6.496	58 ± 4.087	32.5 ± 3.35	49 ± 4.637
Group 2	Day 1	5.4 ± 0.361	17 ± 3.162	52 ± 5.099	35 ± 3.564	34 ± 4.472	61.5 ± 4.77
	Day 2	5 ± 0.365	16 ± 3.082	50 ± 5.196	35 ± 4.062	34.5 ± 4.72	60 ± 4.123
	Day 3	5 ± 0.402	17 ± 2.739	50 ± 5.05	34 ± 4.301	35 ± 4.472	63 ± 3.162
Group 3	Day 1	4.3 ± 0.348	23 ± 3.674	61 ± 4.472	43 ± 4.848	28.9 ± 4.27	52.3 ± 4.31
	Day 2	4 ± 0.374	20 ± 4.416	60 ± 3.937	42 ± 4.583	29 ± 4.062	52 ± 4.183
	Day 3	4 ± 0.43	21 ± 4.301	62 ± 4.301	44 ± 5.196	29.2 ± 3.7	51 ± 5.244

Values are the mean of five replicates with ± SD

Bn = bilirubin, Alb = albumin, TP = total protein.

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 100 mg/kg silymarin)

Group 3 = Normal tested (received 200 mg / kg plant extract)

Table 34. Effect of *E. granulata* extracts on liver health markers in CCl_4 treated rabbits.

Exp. gp	Levels of liver health markers during experiment						
	Interval	Bn (µmol/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)	Alb (g/dl)	TP (g/dl)
Group 4	Day 1	7 ± 0.797	6.7 ± 0.64	270 ± 11.4	115 ± 11.7	17.5 ± 3.84	42.5 ± 5.85
	Day 2	7.2 ± 0.495	6.5 ± 0.667	270 ± 11.07	117 ± 12.4	15.5 ± 4.5	40 ± 5.431
	Day 3	7.5 ± 0.539	6 ± 0.764	265 ± 13.77	114 ± 11.8	14.5 ± 3.84	40 ± 5.099
Group 5	Day 1	8.2 ± 0.495	6.1 ± 0.886	245 ± 13.28	130 ± 16.1	17 ± 3.386	38 ± 2.739
	Day 2	7.8 ± 0.495	8.2 ± 0.718	219 ± 11.64	117 ± 14.3	20 ± 3.808	42 ± 2.55
	Day 3	7.3 ± 0.458	9 ± 0.667	205 ± 9.354	92 ± 16.54	21 ± 3.391	47 ± 2.55
Group 6	Day 1	7.2 ± 0.914	7.2 ± 0.914	248 ± 13.32	115 ± 11.2	14 ± 3.26	44 ± 6.285
	Day 2	7 ± 0.946	7.5 ± 0.964	220 ± 13.47	107 ± 12.4	16.5± 3.64	50 ± 5.477
	Day 3	7 ± 0.828	7.8 ± 1.03	198 ± 14.85	97 ± 8.602	17.5 ± 3.35	53 ± 5.477

Values are the mean of five replicates with ± SD

Bn = bilirubin, Alb = albumin, TP = total protein.

Group 4 = CCl_4 treated negative Control (received empty capsule shell)

Group 5 = CCl_4 treated positive Control (received 100 mg/kg silymarin)

Group 6 = CCl_4 treated tested (received 200 mg / kg plant extract)

Table 35. Effect of *E. granulata* extracts on liver health markers in preventive rabbits groups.

Exp. gp	Levels of liver health markers during experiment						
	Interval	Bn (µmol/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)	Alb (g/dl)	TP (g/dl)
Group 7	Day 1	6.1 ± 0.893	12.2 ± 2.573	100 ± 10.6	78 ± 6.519	24.5 ± 2.72	41 ± 5.874
	Day 2	6.1 ± 0.864	16.5 ± 1.118	93 ± 10.724	71 ± 7.176	25 ± 2.739	44 ± 5.874
	Day 3	5.8 ± 0.795	17.5 ± 1.5	92 ± 11.576	68 ± 7.583	27 ± 2.121	46 ± 5.385

Values are the mean of five replicates with ± SD, Bn = bilirubin, Alb = albumin, TP = total protein.

Group 7 = Preventive control (received 200 mg / kg plant extract along with CCl_4)

Table 36. Comparative liver health markers before and after administration of CCl_4.

Exp. groups		Liver health markers					
		Bn (µmol/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)	Alb (g/dl)	TP (g/dl)
Group 4	Before	5.45 ± 1.285	17 ± 1.871	52 ± 4.123	37 ± 2.915	33.5 ± 3.041	61.5 ± 3.824
	After	9.9 ± 2.138	7.3 ± 1.992	220 ± 20.61	130 ± 9.487	13.5 ± 3.228	39.5 ± 3.542
	% Eff.	81.6	57	323	251	60	35.8
Group 5	Before	5.4 ± 0.828	16 ± 2.372	71 ± 3.808	45 ± 2.55	30.9 ± 2.309	54.1 ± 2.295
	After	9.7 ± 2.668	5.5 ± 1.942	268 ± 9.192	125 ± 14.58	18.5 ± 1.825	36 ± 4.031
	% Eff.	80	65.6	277	177	40	33.5
Group 6	Before	4.34 ± 0.924	21 ± 3.162	59 ± 3.162	56 ± 3.674	33.3 ± 2.85	52.8 ± 3.539
	After	8.6 ± 1.334	10 ± 2.178	280 ± 6.671	142 ± 7.906	18.0 ± 2.372	42.5 ± 2.5
	% Eff.	98	52.4	374	154	46	19.5
Group 7	Before	3.47 ± 0.579	19 ± 2.55	64 ± 4.123	48 ± 2.55	31.9 ± 2.828	60.5 ± 2.923
	After	4.0 ± 1.079	18.5 ± 2.062	75 ± 4.743	90 ± 4.123	26 ± 2.751	54.5 ± 4.61
	% Eff.	15.2	2.6	17.2	87.5	18.5	9.9

Values are the mean of five replicates with ±SD, Bn = bilirubin, Alb = albumin, TP = total protein and Eff = effect.

Table 37. Effect of *E. prostrata* extracts on liver health markers in healthy rabbits.

Exp. gp	Levels of liver health markers during experiment						
	Interval	Bn (µmol/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)	Alb (g/dl)	TP (g/dl)
Group 1	Day 1	3.5 ± 0.474	15 ± 3.536	60 ± 7.071	36 ± 4.123	30 ± 4.46	57 ± 4.123
	Day 2	3.48 ± 0.415	16 ± 3.808	62 ± 6.083	37 ± 3.578	28 ± 5.037	56 ± 3.859
	Day 3	3.5 ± 0.477	16 ± 4.637	59 ± 5.805	35 ± 3.162	28.5 ± 5.08	56 ± 4.105
Group 2	Day 1	4.4 ± 0.667	24 ± 3.536	63 ± 3.536	44 ± 3.808	29 ± 4.441	52 ± 7.171
	Day 2	4.3 ± 0.587	25 ± 3.674	60 ± 4.472	42 ± 4.528	28 ± 4.785	50 ± 7.28
	Day 3	4.4 ± 0.561	27 ± 3.391	61 ± 4.743	41 ± 4.301	28 ± 4.95	51 ± 7.369
Group 3	Day 1	4.3 ± 0.632	18 ± 3.335	58 ± 4.123	61 ± 6.042	32 ± 4.159	66 ± 4.184
	Day 2	4.5 ± 0.825	17 ± 3.536	55 ± 4.301	56 ± 6.124	30 ± 4.686	65 ± 4.062
	Day 3	4.4 ± 0.682	18 ± 4.373	55 ± 4.301	56 ± 6.595	31 ± 4.628	66 ± 4.062

Values are the mean of five replicates with ± SD, Bn = bilirubin, Alb = albumin, TP = total protein

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 100 mg/kg silymarin)

Group 3 = Normal tested (received 200 mg / kg plant extract)

Table 38. Effect of *E. prostrata* extracts on liver health markers in CCl_4 treated rabbits.

Exp. gp	Levels of liver health markers during experiment						
	Interval	Bn (µmol/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)	Alb (g/dl)	TP (g/dl)
Group 4	Day 1	9.9 ± 2.118	7.3 ± 1.992	220 ± 20.61	130 ± 9.49	13.5 ± 3.23	40 ± 3.542
	Day 2	10.2 ± 2.38	8.0 ± 1.606	204 ± 17.33	122 ± 6.96	13 ± 2.85	38 ± 4.09
	Day 3	10.4 ± 2.44	8.2 ± 1.456	190 ± 12.67	115 ± 9.25	13 ± 2.871	37 ± 3.349
Group 5	Day 1	9.7 ± 2.668	5.5 ± 1.942	268 ± 9.192	125 ± 14.58	18.5 ± 1.82	36 ± 4.031
	Day 2	9.2 ± 2.108	7 ± 1.975	252 ± 3.493	118 ± 11.55	19 ± 1.527	37 ± 3.651
	Day 3	8.6 ± 1.908	7.8 ± 2.123	245 ± 8.337	113 ± 13.04	19 ± 1.653	37 ± 3.536
Group 6	Day 1	8.6 ± 1.334	10 ± 2.178	280 ± 6.671	142 ± 7.91	18 ± 2.372	42.5 ± 2.5
	Day 2	8.2 ± 1.092	12.5 ± 2.151	262 ± 3.674	136 ± 8.28	19 ± 2.372	44 ± 1.581
	Day 3	8 ± 1.238	14 ± 1.447	247 ± 6.124	133 ± 8.75	18 ± 1.904	43 ± 2.0

Values are the mean of five replicates with ± SD, Bn = bilirubin, Alb = albumin, TP = total protein

Group 4 = CCl_4 treated negative Control (received empty capsule shell)

Group 5 = CCl_4 treated positive Control (received 100 mg/kg silymarin)

Group 6 = CCl_4 treated tested (received 200 mg / kg plant extract)

Table 39. Effect of *E. prostrata* extracts on liver health markers in preventive rabbits groups.

Exp. gp	Levels of liver health markers during experiment						
	Interval	Bn (µmol/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)	Alb (g/dl)	TP (g/dl)
Group 7	Day 1	4 ± 1.079	18.5 ± 2.062	75 ± 4.743	90 ± 4.123	26 ± 2.751	54.5 ± 4.61
	Day 2	4 ± 1.118	20 ± 2.828	72 ± 5.099	83 ± 4.301	26.8 ± 2.96	55 ± 4.301
	Day 3	3.8 ± 1.056	21 ± 2.915	70 ± 5.196	81 ± 4.062	27 ± 2.739	55 ± 4.637

Values are the mean of five replicates with ± SD, Bn = bilirubin, Alb = albumin, TP = total protein

Group 7 = Preventive control (received 200 mg / kg plant extract along with CCl_4)

Table 40. Effects of ethanolic extracts of *E. granulata* on bone health markers in rabbits.

Exp. gps	Bone health markers before (day 1) and after the treatment					
	Parameter	Day 1	Day 5	Day 10	Day 15	Day 20
Group	ALP (IU/L)	25 ± 1.304	26 ± 0.837	25 ± 1.581	24 ± 0.707	25 ± 1.225
	Cal. (mg/dl)	7.5 ± 0.141	7.4 ± 0.148	7.4 ± 0.122	7.5 ± 0.1	7.5 ± 0.158
	Phos. (mmol/L)	3.2 ± 0.164	3.4 ± 0.158	3.3 ± 0.084	3.4 ± 0.13	3.3 ± 0.158
Group	ALP (IU/L)	32 ± 9.176	34 ± 8.899	36 ± 8.614	35 ± 8.602	36 ± 8.093
	Cal. (mg/dl)	7.4 ± 0.976	7.8 ± 0.95	8.5 ± 1.178	8.8 ± 1.217	8.8 ± 1.194
	Phos. (mmol/L)	4.0 ± 0.397	4.8 ± 0.518	4.9 ± 0.434	4.8 ± 0.385	4.9 ± 0.365
Group	ALP (IU/L)	36 ± 8.319	40 ± 8.044	41 ± 8.124	41 ± 8.585	40 ± 8.258
	Cal. (mg/dl)	5.2 ± 0.618	5.5 ± 0.618	5.6 ± 0.495	5.5 ± 0.619	5.7 ± 0.532
	Phos. (mmol/L)	3.8 ± 0.303	4.3 ± 0.552	4.2 ± 0.484	4.2 ± 0.394	4.2 ± 0.427
Group	ALP (IU/L)	32 ± 6.042	29 ± 5.831	31 ± 6.496	30 ± 6.595	32 ± 5.718
	Cal. (mg/dl)	6.1 ± 1.167	6.4 ± 1.083	5.8 ± 1.021	5.7 ± 1.017	5.7 ± 0.919
	Phos. (mmol/L)	4.2 ± 0.402	4.42 ± 0.593	4.0 ± 0.336	3.8 ± 0.286	3.9 ± 0.259
Group	ALP (IU/L)	36 ± 6.38	42 ± 6.686	43 ± 6.87	41 ± 7.12	42 ± 7.629
	Cal. (mg/dl)	5.7 ± 1.171	6.2 ± 1.27	6.4 ± 1.297	6.3 ± 1.234	6.4 ± 1.335
	Phos. (mmol/L)	4.3 ± 0.559	4.8 ± 0.593	4.7 ± 0.485	4.9 ± 0.54	4.8 ± 0.539
Group	ALP (IU/L)	22 ± 4.817	24 ± 4.658	25 ± 5.119	25 ± 5.718	25 ± 5.357
	Cal. (mg/dl)	7.2 ± 0.78	7.5 ± 0.594	7.6 ± 0.54	7.5 ± 0.719	7.4 ± 0.754
	Phos. (mmol/L)	5.1 ± 0.579	5.3 ± 0.579	5.4 ± 0.472	5.3 ± 0.497	5.5 ± 0.277

Values are mean of five replicates with ± SD, ALP = Alkaline phosphatase, Ca = calcium, P= Phosphates

Group 1 = Normal negative Control (received empty capsule shell daily)

Group 2 = Normal positive Control (received 15µg/kg PTH daily)

Group 3 = Normal tested (received 200mg/kg of EG extract)

Group 4 = Osteoporosis induced –ive control (received 3.12mg/kg dexamethasone daily)

Group 5 = Osteoporosis induced +ive control (received 3.12mg/kg dexamethasone and 15µg/kg PTH daily)

Group 6 = Osteoporosis induced tested (received 3.12mg/kg dexamethasone and 200mg/kg of EG extract daily)

Table 41. Effects of ethanolic extracts of *E. prostrata* on bone health markers in rabbits.

Exp. gps	Bone health markers before (day 1) and after the treatment					
	Parameter	Day 1	Day 5	Day 10	Day 15	Day 20
Group	ALP (IU/L)	32 ± 1.304	31 ± 1.225	31 ± 0.707	30 ± 1.225	32 ± 1.304
	Cal. (mg/dl)	6.1 ± 0.482	6.2 ± 0.354	6.1 ± 0.482	6.0 ± 0.444	6.1 ± 0.444
	Phos. (mmol/L)	4.2 ± 0.541	4.0 ± 0.497	4.2 ± 0.579	4.1 ± 0.449	4.0 ± 0.451
Group	ALP (IU/L)	36 ± 1.304	40 ± 2.121	43 ± 1.304	46 ± 2.168	45 ± 1.871
	Cal. (mg/dl)	4.7 ± 0.415	5.2 ± 0.385	5.5 ± 0.3	5.4 ± 0.297	5.5 ± 0.251
	Phos. (mmol/L)	3.8 ± 0141	4.3 ± 0.167	4.5 ± 0.148	4.5 ± 0.114	4.4 ± 0.255
Group	ALP (IU/L)	20 ± 1.483	22 ± 1.732	24 ± 1.924	23 ± 2.049	24 ± 2.49
	Cal. (mg/dl)	6.2 ± 0.669	6.5 ± 0.733	6.4 ± 0.652	6.5 ± 0.698	6.5 ± 0.698
	Phos. (mmol/L)	5.1 ± 0.466	5.3 ± 0.396	5.4 ± 0.447	5.6 ± 0.548	5.5 ± 0.412
Group	ALP (IU/L)	23 ± 1.0	21 ± 0.837	23 ± 0.837	22 ± 1.225	23 ± 0.837
	Cal. (mg/dl)	7.7 ± 0.597	8.22 ± 1.158	7.4 ± 0.4	7.5 ± 0.295	7.5 ± 0.332
	Phos. (mmol/L)	3.6 ± 0.303	4 ± 0.187	3.3 ± 0.224	3.4 ± 0.245	3.3 ± 0.3
Group	ALP (IU/L)	30 ± 1.871	32 ± 2.345	36 ± 1.483	35 ± 0.837	36 ± 1.304
	Cal. (mg/dl)	7.2 ± 1.665	7.8 ± 1.463	8.2 ± 1.522	8.3 ± 1.462	8.2 ± 1.506
	Phos. (mmol/L)	4.0 ± 0.351	4.8 ± 0.46	4.9 ± 0.356	4.8 ± 0.455	4.9 ± 0.458
Group	ALP (IU/L)	26 ± 1.304	28 ± 0.837	30 ± 0.837	30 ± 1.095	31 ± 1.225
	Cal. (mg/dl)	8.2 ± 1.319	8.5 ± 1.344	8.6 ± 1.187	8.5 ± 1.237	8.7 ± 1.268
	Phos. (mmol/L)	4.0 ± 0.416	4.4 ± 0.406	4.4 ± 0.365	4.4 ± 0.43	4.4 ± 0.472

Values are mean of five replicates with ± SD, ALP = Alkaline phosphatase, Ca = calcium, Phosphates

Group 1 = Normal negative Control (received empty capsule shell daily)

Group 2 = Normal positive Control (received 15µg/kg PTH daily)

Group 3 = Normal tested (received 200mg/kg of EP extract)

Group 4 = Osteoporosis induced –ive control (received 3.12mg/kg dexamethasone daily)

Group 5 = Osteoporosis induced +ive control (received 3.12mg/kg dexamethasone and 15µg/kg PTH daily)

Group 6 = Osteoporosis induced tested (received 3.12mg/kg dexamethasone and 200mg/kg of EP extract daily)

Table 42. Effects of *E. granulata* extract (ethanolic) on electrolytes profile in rabbits.

Exp. groups		Electrolyte profile before and after administering the plant extract		
		Na (mmol/l)	K (mg/dl)	Cl (mmol/l)
Group 1	Before	138 ± 7.727	4.3 ± 0.577	102 ± 4.528
	After	139 ± 3.962	4.44 ± 0.23	101 ± 5.385
Group 2	Before	145 ± 5.385	4.4 ± 0.212	105 ± 4.472
	After	124 ± 2.55	5.5 ± 0.412	96 ± 4.123
Group 3	Before	136 ± 4.743	4.9 ± 0.292	111 ± 3.05
	After	137 ± 2.236	5.0 ± 0.539	110 ± 4.123
Group 4	Before	139 ± 2.236	5.2 ± 0.474	103 ± 2.915
	After	139 ± 3.536	5.1 ± 0.316	102 ± 4.743
Group 5	Before	143 ± 3.536	5.7 ± 0.158	106 ± 4.123
	After	140 ± 4.123	5.4 ± 0.474	110 ± 4.472
Group 6	Before	140 ± 2.55	4.8 ± 0.316	103 ± 3.808
	After	138 ± 3.162	4.8 ± 0.412	101 ± 6.042

Values or the mean of five replicates with ± SD

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 0.5mg / kg frusemide)

Group 3 = Normal tested (received 50 mgs / kg plant extract for five days)

Group 4 = Normal tested (received 100 mgs / kg plant extract for five days)

Group 5 = Normal tested (received 150 mgs / kg plant extract for five days)

Group 6 = Normal tested (received 200 mgs / kg plant extract for five days)

Table 43. Effects of *E. prostrata* extract (ethanolic) on electrolytes profile in rabbits.

Exp. groups		Electrolyte profile before and after administering the plant extract		
		Na (mmol/l)	K (mg/dl)	Cl (mmol/l)
Group 1	Before	133 ± 2.236	5.3 ± 0.381	113 ± 2.55
	After	130 ± 4.123	5.4 ± 0.46	111 ± 5.099
Group 2	Before	135 ± 3.162	4.1 ± 0.158	104 ± 5.099
	After	119 ± 2.236	4.5 ± 0.381	96 ± 4.743
Group 3	Before	140 ± 2.915	4.3 ± 0.412	111 ± 5.099
	After	138 ± 2.55	4.2 ± 0.255	111 ± 4.324
Group 4	Before	133 ± 4.123	4.7 ± 0.632	102 ± 5.099
	After	134 ± 2.55	4.8 ± 0.728	103 ± 6.325
Group 5	Before	144 ± 3.808	5.1 ± 0.539	100 ± 2.915
	After	142 ± 4.123	4.8 ± 0.316	101 ± 6.042
Group 6	Before	132 ± 1.414	5.5 ± 0.51	111 ± 4.528
	After	132 ± 2.236	5.0 ± 0.515	112 ± 1.414

Values or the mean of five replicates with ± SD

Group 1 = Normal negative Control (received empty capsule shell)

Group 2 = Normal positive Control (received 0.5mg / kg frusemide)

Group 3 = Normal tested (received 50 mgs / kg plant extract for five days)

Group 4 = Normal tested (received 100 mgs / kg plant extract for five days)

Group 5 = Normal tested (received 150 mgs / kg plant extract for five days)

Group 6 = Normal tested (received 200 mgs / kg plant extract for five days)

10. Effects on hematology

1. Antianemic activity

***Euphorbia granulata* Forssk**

The impact after 3 days of treatment with phenylhydrazine (PHZ) is presented in Table 44. The therapeutic effect of standard anti-anemic preparation and plant extract in normal and anemic rabbits are summarized in Table 45. Group 7 was treated with extract along with administration of PHZ. The preventive effects of plant extracts are given in Table 45.

Comparison of rabbits after with Phenylhydrazine (PHZ): To produce anemic rabbits phenylhydrazine (PHZ) was administered to group 4, 5 and 6. PHZ is a toxic molecule the exposure of which results in Rbcs damage and anemia (Berger, 2007). This molecule was used to produce anemic rabbits for evaluating anti-anemic potential of *E. granulata*. Three days treatment with PHZ produced rabbits with deranged hematological values. On day 3 the values of Rbcs, Hb and PCV decreased significantly ($p<0.001$) compared to zero time values, are shown in Table 44. Comparing the values of group 4 only reveals that Rbcs count decreased by 43.5% from 4.6 (± 0.474) to 2.6 (± 0.381). Similarly Hb decreased by 45.4% from 10.8 (± 0.57) to 5.9 (± 0.51) and PCV decreased by 47% from 32.5 (± 1.501) to 17.2 (± 1.299).

Comparison of normal rabbits: Negative control group maintained normal level of Rbcs, Hb and PCV during five days of experiment. Group 2 rabbits showed identical response with Vitamin B_{12} and iron preparation, as of negative control. The possible reason was that the group had already healthy physiology and was with normal hematological levels. Therefore no increase or decrease was noted for Rbcs, Hb and PCV. As the other parameters (MCH, MCHC and MCV) are linked to the first three, they don't need to be calculated in absence of change in the first. Application with *E. granulata* extract showed that Rbcs count was not changed from base line level of 5.7 x 10^6 (±0.54)/L. However, values of Hb increased by 5% from 11.7 (±0.983) to 12.3 (±0.904). PCV enhanced by 5.4% from 35 (±2.55) to 37% (±3.347). With increases in Hb and PCV, the values of MCH, MCHC and MCV also increased (Table 45). Studies show that there is a direct correlation of Rbcs, Hb and PCV' i.e. the change in value of one

influence the other (Ikpi & Nku, 2008). The slight increase in these values may be due to the stimulant effect on bone marrow to produce more Rbcs with high concentration of hemoglobin. The high iron contents of *E. granulata* (Table 9) may have also contributed to the increased Hb concentration in test rabbits.

The lack of hemoglobin or decrease in RBCs results in anemia. The red cell during anemia cannot meet the need of the tissues demand for oxygen and hence apoxia and death of the tissue. Failure of blood to clot properly leads to blood losses i.e. sickle cell anemia and thalassemia (Lichtman *et al*., 2007). Medicinal plants have shown to improve hemoglobin level in anemia and improve RBC count and PCV (Iwalewa *et al*., 2009; Oluwole *et al*., 2009; Nwinuka *et al*, 2008). In the present study the main focus was on the impact of plant extract on red blood cells count (Rbcs), hemoglobin (Hb) and hematocrit or packed cell volume (PCV). These parameters are indicative of anemia (Iwalewa *et al*., 2009). Other parameters including mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) was calculated from the data of the first three parameters. The results obtained with ethanolic extract of *E. granulata* are encouraging in treating anemic patients.

Comparison of anemic rabbits: In anemic set of rabbits group 4 was kept as negative control. The levels of RBCs, Hb and PCV remained unchanged up till fifth day of experiment. Treating group 5 with vitamin B_{12} and iron preparations revealed that the level of RBCs improved by 26% from 2.9 (± 0.292) to 3.6 (±0.51). Berger (2007) pointed that iron preparations modulate the regeneration response; the present results are in complete agreement with this statement. Rabbits in group 5 received 200 mg of plant extract, where the RBCs count increased by 38% from 2.9 (± 0.381) to 4 (± 0.412). In vitamin B_{12} and iron preparations treated group the level of Hb improved by 7.8% from 7.7 (± 0.474) to 8.3 (± 0.57) and PCV 5.5% from 23.5 (± 1.119) to 24.8 (± 1.643). In case of extract treated group Hb enhanced by 33.9% from 6.2 (± 0.922) to 8.3 (± 0.608) and PCV by 30.9% from 18.8 (± 2.864) to 24.6 (± 1.797). Calculating the values of MCH, MCHC and MCV for positive control group reveals that MCH decreased by 14.7% from 27.2 (± 3.39) to 23.2 (± 2.05) and MCV by 13.5% from 81 (± 9.909) to 70 (± 6.813). This trend of decrease in MCH and MCV but enhanced RBCs count and Hb levels suggests

that fresh RBCs are produced but with smaller size and smaller Hb concentration. Hence a natural regeneration process started taking help from iron and B_{12} supplement. Comparing these values for extract treated group reveals that MCH, MCHC and MCV had not changed with extracts. Increase in values of RBCs, Hb and PCV and no change in MCH, MCHC and MCV indicates that RBCs are produced having normal size and optimum Hb concentration. This further suggests that the plant extract contained some novel ingredients or combination of factors that not only speed up the RBCs production but also with good amount of hemoglobin.

Comparison of preventive group: Co-administration of both plant extract and PHZ had no protective effect on the overall hematology markers. As discussed above normal rabbits when treated with PHZ, anemia was produced. Administering PHZ along with plant extracts also resulted in anemia which is revealed from the results. Table 44 shows that RBCs, Hb and PCV values of these rabbits significantly ($p<0.001$) decreased compared to the time before administration of PHZ. The value of RBCs decreased by 48% from 5.8 (±0.381) to 3(± 0.321), Hb by 49% from 14.6 (± 0.632) to 7.4 (± 0.474) and PCV by 50% from 44 (± 1.924) to 22 (± 1.632). However the values of MCH, MCHC and MCV sustained. This suggests that the plant extract was not effective in preventing the damage caused by PHZ however the size and normal hemoglobin contents of the cell were sustained. Treatment with PHZ produces anemia by damaging red blood cells. PHZ induces anemia by increasing ROS and decreasing glutathione (GSH). With increased ROS increased binding of denatured hemoglobin to membrane cytoskeleton occur which results in hemolytic injury. Hence PHZ induced anemia is not due to change in membrane lipids but by changes in RBCs protein (Berger, 2007). The extract might have the ability to keep these proteins from the damaging effect of PHZ. Five days treatment with plant extract improves the levels of all hematology parameters at the pattern of that noted for the anemic set of rabbits.

The results suggest the positive role of plant extract in treating anemia.

***Euphorbia prostrata* Aiton**

E. prostrata was tested for anti-anemic potential. The rabbits were administered PHZ with the results in Table 46. The impact of anti-anemic preparation and plant extract

on normal, anemic and preventive control rabbits are given in Table 47. The results obtained for *E. prostrata* are compared with *E. granulata*.

Comparison of rabbits after with Phenylhydrazine (PHZ): Group 4, 5 and 6 of rabbits were treated with PHZ. Compared to the base line levels, the values of RBCs, Hb and PCV significantly ($p<0.001$) decreased. The rabbits with highly disturbed hematology values were considered for further study.

Comparison of normal rabbits: Group 1 rabbits were kept as negative control. Blood samples were analyzed at same intervals as that for positive control and test groups. The results are shown in Table 47. Like previous results, no change was observed in hematological markers in this group. Group 2 was treated with anti-anemic preparation (Table 47). Like the previous experiment, RBCs count increased slightly by 7.9% from 4.96 (± 0.77) to 5.34 (± 0.78). Similarly, Hb level raised slightly by 1.5% from 12.8 (± 0.458) to 13 (± 0.396) and PCV improve slightly by 4% from 38 (± 1.949) to 39 (± 1.414). Hematological profile is slightly affected by routine food and water intake. These negligible changes do not seems to be therapeutic effects rather seems routine physiological changes which takes place with water and food intake. Furthermore administering the extract to group 3 (test group) also had not impacted the hematological profile of rabbits. In previous experiment it was observed that *E. granulata* extract induced improvement in hematological profile of even in normal rabbits (group 3). This suggests that, *E. prostrata* is not as *E. granulata* in improving hematological profile of normal rabbits.

Comparison of anemic rabbits: Hematological profile of negative control group showed negligible ups and downs which might be the effects of normal food and water intake. PHZ induced anemic rabbits (group 5) when treated with iron and B_{12} preparation, RBCs count increased by 46.3% from 2.8 (± 0.316) to 3.84 (± 0.288). However, anemic rabbits when treated with plant extract from *E. prostrata*, RBCs count enhanced by 15.6% from 3.2 (± 0.354) to 3.7 (± 0.477). This indicates that *E. prostrata* was not as effective in enhancing RBCs count as *E. granulata*. Furthermore, Hb increased by 28% from 5.4 (± 0.51) to 6.9 (± 0.412) and PCV by 37% from 16.5 (± 1.501) to 22.6 (± 3.879) with drug. In case of plant, with 200 mg extracts the Hb levels increased by 21.8% from

5.5 (± 0.51) to 6.7 (± 0.534) and PCV enhanced by 26% from 16.5 (± 1.503) to 20.8 (± 1.643). The large improvement in hematological profile with iron and B_{12} preparation and small changes with plant extract suggest the small role of plant in treating anemia. These results consolidate the statement that iron preparations modulate the regeneration from anemia (Berger. 2007). Further data for positive control group reveals that MCH decreased by 6.7% from 19.3 (± 0.432) to 18 (± 0.329). MCHC had not change while MCV decrease by 8.5% from 59 (± 2.366) to 54 (± 1.383). These investigations once again support the previous experiment results that with iron and B_{12} the resulted RBCs were small in size and had little amount of hemoglobin. The results of treatment of group 6 with *E. prostrata* extract are shown in Table 47. These results suggests that although *E. prostrata* did not affect the levels of hematological parameters in group 2 (normal rabbits) but was found effective in case of anemic rabbits. This further creates the idea that the plant is therapeutically effective in diseased subject where the effect is needed but has no effect in normal subject where it is not needed. The values obtained for MCH, MCHC and MCV differentiate the affect from standard preparation that here the data suggest normal size of RBCs with more Hb. Comparing the effects with *E. granulata* shows that *E. granulata* enhanced the hematological parameters both in normal rabbits (group3) and anemic (group 6) but *E. prostrata* is found effective only in anemic subject.

Comparison of preventive group: Table 46 shows that, like the experiment with *E. granulata* the plant extract could not prevent the damage caused by PHZ in group 7 rabbits. But same group when continuously treated with plant extract resulted in improvement of hematological profile (Table 47) on the pattern of group 6.

The results suggest the use of *E. granulata* both in anemic and prophylactic conditions while *E. prostrata* might be used in anemic conditions only.

2. Immuno-modulatory activity

Euphorbia granulata **Forssk**

Table 48 shows the immunological profile of rabbits before and after treatment with drug and of plant extracts after 5 days (A 5 D).

Reduction in number or disorder in function WBCs leads to weak immunity i.e. AIDs (Cowan, 2012). In immunodeficiency diseases (AIDs) both the cell mediated and antibody mediated immune system fails to prevent microbes from infections. The situation often results in death. The results show that the immunological profile of the negative control rabbits had not changed before and after 5 days (A 5 D) of the experiment. Filgrastim is a glycoprotein that stimulates human granulocytes colony. They are myloids growth factor which stimulates bone marrow to produce more WBCs (Vieira, 2011). Total leukocyte count (TLC) rose by 61% from 9 (± 1.02) to 14.5 (± 0.6)/L with filgrastim in group 2. Medicinal plants have shown positive impact on T-lymphocytes and B-Lymphocytes and improvement in leukemia (Illmer *et al*., 2005). Enhancing effect on WBCs number and activity has also been observed (Akah *et al*., 2009; Oluwole *et al*., 2009; Taiwo *et al.*, 2009). Immunoregulatory effect is also noted for medicinal plants (Winkler *et al.*, 2005). Groups 3, 4, 5 and 6 received 50, 100, 150 and 200 mg/kg plant extract respectively but none had produced change in TLC. Neutrophils are granulocyte white blood cells. The count of neutrophils increased by 19.5% from 41 (± 4.12) to 49% (± 2.23) with drug. A slight decrease of 5.1% in neutrophils count from 39 (± 3.8) to 37 (± 3.16) was noted at 100 mg of extract. However this decrease was statistically insignificant. A significant decrease might have been considered as immune-suppressant activity of plant. At 150 of extract an increase of neutrophils count from 42 (± 3.16) to 50 (± 0.381) was observed. There was a 19% increase. Considering these results, 100 mg had slight immune-suppressant effect while 150 mg seems to be an immune-stimulant dose. All other doses of plant extract were ineffective to produce change in number of neutrophils. Lymphocytes are agranulocyte white blood cell. In positive control group of rabbits, lymphocytes decreased by 14.3% from 56 (± 4.637) to 48% (± 2.864). In case of plant extract, 150 mg was more effective where the lymphocytes count decreased from 54 (±3 .347) to 46 (± 2.702). There was 14.8% decrease of lymphocytes. However, none of the doses of plant extract was active to change the value from base line level. Normal ranges of eosinphils, basophils or monocytes were neither affected with filgrastim nor with doses of *E. granulata* extract. The platelets (Plts) or thrombocyts prevent bleeding in injury by forming clot. Improper

function of the thrombocytes results in blood loss and death e.g. hemophilia. Platelets count was sustained in positive control group till 5th day of procedure. However with 100 mg plant extract Plts decreased negligibly by 1.3%, from 385 (± 20.61) to 380 (± 41.83). The increase in neutrophils and decrease in lymphocytes occurred with 150 mg only. This effect was not noted with 200 mg/kg of extract. This suggests that not quantity of dose but ratio was important. Immuno-regulatory effect if any could be associated with 150 mg/kg body weight of extract.

It is concluded that the plant extracts had no effect on the total leukocyte count (TLC). In differential leukocytes counts (DLC) neutrophils were noted to enhance with 150 mg dose. Some variation in lymphocytes was also noted at 150 mg but statistically these were not significant. The reason might be that the phytochemicals found in *E. granulata* extract have no constituents to effect the production, differentiation and proliferation of WBCs. The results with other doses are not supporting either immune-stimulant or immune-suppressant potential of plant.

***Euphorbia prostrata* Aiton**

The results obtained with filgrastim and different extract doses on the TLC and DLC of rabbits are shown in Table 49.

The present experiment was set for the immune-stimulant or immune-suppressant role of *E. prostrata*. For this experiment group 1 and 2 rabbits received the same treatments as before' i.e. no drug in first case and treatment of filgrastim in second case. In both situations identical results with previous experiment were achieved. In negative control group no change occurred in TLC or DLC while with filgrastim, TLC, neutrophils increased and lymphocytes decrease. Table 49 shows that at 100 mg extract TLC increased from 7.5 (± 0.492) to 8 (± 0.922). This was 6.7% increase. On the other hand at 200 mg the TLC decreased by 13% from 11.5 (± 0.54) to 10 (± 1.02). Increase in number at one dose and decrease at the other is beyond understanding but possibly could have some relation with plant ingredients. On the other hand the count of neutrophils decreased with all doses of extract in a haphazard way. With 50 mg extract neutrophils count decreased from 44 (± 1.58) to 42 (± 3.54), a 12% decrease. At 100 mg extract the decrease was 5.1% from 39 (± 3.49) to 37 (± 3.16). Increasing the dose to 150 mg

dropped the count by 7.4% from 54 (± 1.41) to 50 (± 1.0). At 200 mg the count dropped by 6% from 50 (± 1.58) to 47 (± 1.58). Surprisingly significant decrease (12%) was observed at low dose (50 mg) but with high dose (200 mg) only 6% decrease recorded. A proportionate increase in lymphocytes was noted at all doses of extracts. The table shows that at 50 mg of extracts the number of lymphocytes increased by 3.8% from 52 (± 1.817) to 54 (± 5.128). Increasing the extract dose to 100 mg resulted in an increase of 3.5% from 57 (± 4.062) to 59 (± 4.472). Similarly, at 150 mg the lymphocytes count increased by 9.5% from 42 (± 1.871) to 46 (± 1.789). At high dose of 200 mg of extract the count rose by 6.5% from 46 (± 2.28) to 49 (± 2.702). Here the increases in lymphocyte count increases with dose from 3.8% with 50 mg to 9.5% with 150 mg. The 6.5% increase with 200 mg was again an enigma. Eosinophils, basophils, monocytes and platelets were not affected with all extract doses (50, 100, 150 and 200 mg/kg).

The results indicated that the plant have some unique combination of ingredients which stimulate bone marrow for WBC production or differentiation in a very unique way.

Table 44. Hematological markers of rabbits before and 4 days after administering phenylhydrazine.

Exp. groups		Hematological markers					
		Rbcs(10^6) / l	Hb (g/dl)	PCV (%)	MCH	MCHC	MCV
Group 4	Before	4.6 ± 0.474	10.8 ± 0.57	32.5 ± 1.501	23.8 ± 3.725	33.3 ± 1.029	71.4 ± 10.55
	After	2.6 ± 0.381	5.9 ± 0.51	17.2 ± 1.299	22.8 ± 1.508	34.2 ± 0.689	67 ± 5.595
Group 5	Before	5.1 ± 0.922	13.3 ± 0.863	40.8 ± 3.273	26.5 ± 3.392	32.6 ± 0.591	81.3 ± 9.114
	After	2.9 ± 0.292	7.7 ± 0.474	23.5 ± 1.119	27.2 ± 3.39	32.8 ± 0.507	81 ± 9.909
Group 6	Before	5.6 ± 0.51	12.3 ± 0.982	36.8 ± 2.861	22 ± 0.891	33.4 ± 0.244	65.7 ± 2.841
	After	2.9 ± 0.381	6.2 ± 0.922	18.8 ± 2.864	21.3 ± 0.696	33 ± 0.621	65 ± 2.084
Group 7	Before	5.8 ± 0.381	14.6 ± 0.632	44 ± 1.924	25 ± 0.581	33.3 ± 0.25	75.6 ± 1.703
	After	3 ± 0.321	7.4 ± 0.474	22 ± 1.632	24.3 ± 1.022	33.4 ± 0.661	69 ± 7.569

Values are the mean of five replicates with ± SD

Table 45. Effect of *Euphorbia granulata* extract on hematological markers.

Exp. groups		Hematological markers					
		Rbcs(10^6) / l	Hb (g/dl)	PCV (%)	MCH	MCHC	MCV
Gro	Before	5.8 ± 0.698	12.7 ± 0.828	38.8 ± 2.864	22.3 ± 3.448	32.7 ± 0.643	68 ± 10.084
	After	5.8 ± 0.561	12.6 ± 0.829	39 ± 2.864	22 ± 2.843	32.6 ± 0.496	67 ± 8.45
Gro	Before	6 ± 1.363	12.3 ± 1.041	37.5 ± 3.042	21.8 ± 6.826	32.9 ± 0.164	66 ± 20.492
	After	5.9 ± 1.159	12.3 ± 1.043	37 ± 3.0	21.6 ± 5.921	33.2 ± 0.392	65 ± 17.341
Gro	Before	5.7 ± 0. 54	11.7 ± 0.983	35 ± 2.55	20.5 ± 0.886	33.5 ± 0.85	62 ± 1.657
	After	5.7 ± 0.667	12.3 ± 0.904	37 ± 3.347	21.6 ± 1.147	33 ± 1.031	66 ± 3.614
Gro	Before	2.6 ± 0.381	5.9 ± 0.51	17.2 ± 1.299	22.8 ± 1.508	34.2 ± 0.689	67 ± 5.595
	After	2.6 ± 0.43	5.9 ± 0.447	17.2 ± 1.299	23 ± 2.158	34.2 ± 0.802	67 ± 5.991
Gro	Before	2.9 ± 0.292	7.7 ± 0.474	23.5 ± 1.119	27.2 ± 3.39	32.8 ± 0.507	81 ± 9.909
	After	3.6 ± 0.51	8.3 ± 0.57	24.8 ± 1.643	23.2 ± 2.05	33.5 ± 0.659	70 ± 6.813
Gro	Before	2.9 ± 0.381	6.2 ± 0.922	18.8 ± 2.864	21.3 ± 0.696	33 ± 0.621	65 ± 2.084
	After	4 ± 0.412	8.3 ± 0.608	24.6 ± 1.797	20.8 ± 0.644	33.6 ± 0.456	62 ± 2.32
Gro	Before	3 ± 0.321	7.4 ± 0.474	22 ± 1.632	24.3 ± 1.022	33.4 ± 0.661	69 ± 7.569
	After	3.7 ± 0.412	9.2 ± 0.381	27.4 ± 1.126	25 ± 1.791	33.5 ± 0.185	75 ± 5.489

Values are the mean of five replicates with ± SD

Group 1 = Normal negative Control

Group 2 = Normal positive Control

Group 3 = Normal tested

Group 4 = phenylhydrazine-HCl treated negative Control

Group 5 = phenylhydrazine-HCl treated positive Control

Group 6 = phenylhydrazine-HCl treated tested

Group 7 = Preventive control

Table 46. Hematological markers of rabbits before and 4 days after administering phenylhydrazine.

Exp. groups		Hematological markers before and after 4 days treatment					
		Rbcs(10^6) / l	Hb (g/dl)	PCV (%)	MCH	MCHC	MCV
Group 4	Before	5.8 ± 0.354	13.7 ± 0.51	41 ± 1.584	23.6 ± 0.682	33.4 ± 0.179	71 ± 2.248
	After	3.1 ± 0.412	5.9 ± 0.381	17.8 ± 1.493	19.2 ± 1.386	33 ± 0.864	58 ± 3.596
Group 5	Before	4.7 ± 0.354	10.4 ± 0.667	31.6 ± 2.074	22.1 ± 0.28	33 ± 0.238	67 ± 0.936
	After	2.8 ± 0.316	5.4 ± 0.51	16.5 ± 1.501	19.3 ± 0.432	33 ± 0.611	59 ± 2.366
Group 6	Before	6.1 ± 0.404	13.6 ± 0.608	41 ± 1.802	22.1 ± 0.521	33.2 ± 0.296	68 ± 1.864
	After	3.2 ± 0.354	5.5 ± 0.51	16.5 ± 1.503	17.2 ± 0.665	33 ± 0.635	52 ± 1.305
Group 7	Before	5.3 ± 0.57	12 ± 0.632	36 ± 1584	22.6 ± 1.285	33.2 ± 0.302	68 ± 4.4
	After	2.8 ± 0.316	5 ± 0.224	15.1 ± 0.792	18 ± 1.604	33 ± 0.91	55 ± 5.642

Values are the mean of five replicates with ± SD

Table 47. Effect of *Euphorbia prostrata* extract on hematological markers.

Exp. groups		Hematological markers before and after 4 days treatment					
		Rbcs(10^6) / l	Hb (g/dl)	PCV (%)	MCH	MCHC	MCV
Gro	Before	5.7 ± 0.667	13 ± 0.746	39 ± 3.174	23 ± 1.632	33.4 ± 0.929	69 ± 4.248
	After	5.7 ± 0.497	13 ± 0.589	39 ± 3.174	23 ± 1.069	33.3 ± 1.513	68 ± 3.105
Gro	Before	4.96 ± 0.77	12.8 ± 0.458	38 ± 1.949	26.2 ± 3.613	33.4 ± 0.577	79 ± 9.822
	After	5.34 ± 0.78	13 ± 0.396	39 ± 1.414	24.7 ± 2.995	33.4 ± 0.278	74 ± 8.418
Gro	Before	5.3 ± 0.917	12.5 ± 0.469	37 ± 1.342	24 ± 3.977	33 ± 0.173	72 ± 12.02
	After	5.3 ± 0.926	12.5 ± 0.498	37.6 ± 1.673	24.2 ± 4.2	33 ± 0.404	73 ± 11.78
Gro	Before	3.1 ± 0.412	5.9 ± 0.381	17.8 ± 1.493	19.2 ± 1.386	33 ± 0.864	58 ± 3.596
	After	3.14 ± 0.451	6 ± 0.472	18.4 ± 15.17	19.4 ± 1.617	33 ± 0.484	59 ± 5.324
Gro	Before	2.8 ± 0.316	5.4 ± 0.51	16.5 ± 1.501	19.3 ± 0.432	33 ± 0.611	59 ± 2.366
	After	3.84 ± 0.288	6.9 ± 0.412	22.6 ± 3.879	18 ± 0.329	33.5 ± 1.045	54 ± 1.383
Gro	Before	3.2 ± 0.354	5.5 ± 0.51	16.5 ± 1.503	17.2 ± 0.665	33 ± 0.635	52 ± 1.305
	After	3.7 ± 0.477	6.7 ± 0.534	20.8 ± 1.643	22.4 ± 2.364	33 ± 0.321	67 ± 6.967
Gro	Before	2.8 ± 0.316	5 ± 0.224	15.1 ± 0.792	18 ± 1.604	33 ± 0.91	55 ± 5.642
	After	3.3 ± 0.158	8.5 ± 0.51	25.4 ± 1.501	25.7 ± 0.358	33.3 ± 0.397	77 ± 1.568

Values are the mean of five replicates with ± SD

Group 1 = Normal negative Control

Group 2 = Normal positive Control

Group 3 = Normal tested

Group 4 = phenylhydrazine-HCl treated negative Control

Group 5 = phenylhydrazine-HCl treated positive Control

Group 6 = phenylhydrazine-HCl treated tested

Group 7 = Preventive control

Table 48. Effects of *Euphorbia granulata* extract on immunological markers of rabbits.

Exp. groups	Immunological markers before and after 5 days treatment						
	Tlc (10^9) / L	Neu (%)	Eos (%)	Bas (%)	Mon (%)	Lymph (%)	Plts 10^9 L^{-1}
Group 1	11 ± 0.949	43 ± 3.8	1.5 ± 0.35	0.5 ± 0.33	3.5 ± 0.5	51 ± 3.912	360 ± 57
A 5 D	10.3 ± 2.02	42 ± 2.55	1.2 ± 0.67	0.6 ± 0.34	3 ± 0.354	53 ± 2.588	360 ± 31.62
Group 2	9 ± 1.02	41 ± 4.12	0.5 ± 0.61	0.7 ± 0.07	2 ± 0.354	56 ± 4.637	450 ± 15.81
A 5 D	14.5 ± 0.60	49 ± 2.23	0.5 ± 0.35	0.6 ± 0.25	2 ± 0.5	48 ± 2.864	448 ± 43.17
Group 3	10.5 ± 0.6	44 ± 4.12	1 ± 0.791	0.7 ± 0.19	2.5 ± 0.5	52 ± 4.817	410 ± 31.62
A 5 D	10 ± 0.474	42 ± 2.23	1 ± 0.354	0.9 ± 0.05	2.6 ± 0.96	54 ± 2.55	410 ± 41.23
Group 4	7.6 ± 0.903	39 ± 3.8	1.5 ± 0.35	0.5 ± 0.0	1.5 ± 0.35	57 ± 4.147	385 ± 20.61
A 5 D	8 ± 0.886	37 ± 3.16	1.2 ± 0.67	0.5 ± 0.33	1.5 ± 0.79	59 ± 3.209	380 ± 41.83
Group 5	10 ± 0.632	42 ± 3.16	1 ± 0.0	0.6 ± 0.18	2 ± 0.354	54 ± 3.347	370 ± 38.08
A 5 D	10 ± 0.381	50 ± 2.24	1 ± 0.791	0.5 ± 0.23	2.5 ± 0.79	46 ± 2.702	368 ± 38.34
Group 6	11.5 ± 0.66	50 ± 2.55	1.5 ± 0.5	0.6 ± 0.13	2 ± 0.354	46 ± 2.387	430 ± 15.81
A 5 D	11 ± 0.632	47 ± 2.55	1.5 ± 0.35	0.4 ± 0.4	2 ± 0.612	49 ± 2.121	432 ± 13.04

Values are the mean of five replicates with ± SD

Tlc = Total leukocytes, Neu =neutrophils, Eos = eosinophils, Bas = basophils, Mon = monocytes, Lymph = lymphocytes, Plts = platelets or thrombocytes, A 5 D = after 5 days

Table 49. Effects of *Euphorbia prostrata* extract on immunological markers of rabbits.

Exp. groups	Immunological markers before and after 5 days treatment						
	Tlc (10^9) / L	Neu (%)	Eos (%)	Bas (%)	Mon (%)	Lymph (%)	Plts 10^9 L^{-1}
Group 1	11 ± 0.949	43 ± 3.2	1.5 ± 0.61	0.6 ± 0.2	3.5 ± 0.35	51 ± 2.55	360 ± 31.62
A 5 D	10.5 ± 0.57	42 ± 3.16	1.5 ± 0.35	0.7 ± 0.17	3 ± 0.612	53 ± 3.564	360 ± 41.23
Group 2	9 ± 0.696	41 ± 3.16	0.5 ± 0.61	0.7 ± 0.15	2 ± 0.707	56 ± 2.966	450 ± 31.62
A 5 D	12.5 ± 0.73	49 ± 1.79	0.5 ± 0.5	0.6 ± 0.22	2 ± 0.354	48 ± 3.05	448 ± 62.8
Group 3	10.5 ± 0.7	44 ± 1.58	1 ± 0.5	0.9 ± 0.0	2.5 ± 0.79	52 ± 1.817	410 ± 41.23
A 5 D	10 ± 0.632	42 ± 3.54	1 ± 0.791	0.9 ± 0.0	2.5 ± 0.79	54 ± 5.128	410 ± 41.23
Group 4	7.5 ± 0.492	39 ± 3.49	1.5 ± 0.5	0.5 ± 0.18	1.5 ± 0.78	57 ± 4.062	385 ± 34.82
A 5 D	8 ± 0.922	37 ± 3.16	1.5 ± 0.5	0.5 ± 0.19	1.5 ± 0.79	59 ± 4.472	387 ± 36.5
Group 5	10 ± 1.02	54 ± 1.41	1 ± 0.8	0.5 ± 0.34	2.5 ± 0.61	42 ± 1.871	370 ± 47.43
A 5 D	10 ± 0.474	50 ± 1.0	1 ± 0.79	0.6 ± 0.4	2.5 ± 0.35	46 ± 1.789	368 ± 40.25
Group 6	11.5 ± 0.54	50 ± 1.58	1.5 ± 0.35	0.5 ± 0.38	2 ± 0.791	46 ± 2.28	430 ± 38.08
A 5 D	10 ± 1.02	47 ± 1.58	1.5 ± 0.35	0.5 ± 0.34	2 ± 0.962	49 ± 2.702	428 ± 28.64

Values are the mean of five replicates with ± SD

Tlc = Total leukocytes, Neu =neutrophils, Eos = eosinophils, Bas = basophils, Mon = monocytes, Lymph = lymphocytes, Plts = platelets or thrombocytes, A 5 D = after 5 days

Group 1 = Normal negative Control received empty capsule shell for five days

Group 2 = Normal positive Control (received 10 µg / kg filgrastim for five days)

Group 3 = received 50 mgs/kg plant extract for five days

Group 4 = received 100 mgs/kg plant extract for five days

Group 5 = received 150 mgs/kg plant extract for five days

Group 6 = received 200 mgs/kg plant extract for five days

11. Antioxidant activity

***Euphorbia granulata* Forssk**

The compounds that give electrons are called as antioxidants. Diphenyl picrylhydrazyl (DPPH) is a stable free radical with an odd electron and strong absorbance and 517nm. It can accept an electron or hydrogen from any donor compound and it absorbance is reduced which can be quantitatively measured (Ara & Nur, 2009). The DPPH free radical scavenging ability of whole plant, its roots, stem and leaves are compared with standard antioxidant (ascorbic acid) in Table 50.

Negative control comprised of methanol and plant extract while ascorbic acid were positive control for this experiment. At 1.0 mg / ml of ascorbic acids, 98.59% (± 0.4) quenching of DPPH was noted. At this concentration the whole plant showed 59.93% (± 1.058) activity while leaves exhibited the highest activity of 70.58% (± 0.195). The roots were found with minimum activity of 55.96% (± 1.347). Natural antioxidants are compounds that minimize the injurious effects of reactive free radicals produced during metabolism in plants and animals. They do it by adsorbing and neutralizing, quenching and decomposing (Jimoh *et al*., 2010). In plants phenolic compounds are known for their strong antioxidant activity (Adedapo *et al*., 2009). In the present study the overall results show high activities of the plant and its parts. At 1mg/ml the highest activity was recorded for leaves (70.58% ± 0.195) while roots had least (55.96% ± 1.347). Leaves are metabolically the most active site. They are more prone to the hazards of free radicals therefore it might be an adaptation to overcome the hazards of these damages. Stem is the medium of translocation for these metabolites from leaves to the roots and for this reason slightly higher (57.95% ± 1.924) activity is exhibited than the roots (55.96% ± 1.347). The whole plant shows cumulative activity (59.93% ± 1.058). Therefore, its activity ranged between stem and leaves. The activities showed by different parts of *E. granulata* have high correlation with the chemical composition of the plant. Before undergoing DPPH scavenging activity *Euphorbia granulata* was analyzed for six different phytochemicals. These included total phenolics, flavonoids, alkaloids, tannins, glycosides and saponins. A lot of variation was noted for these chemicals from one part to another. The leaves were rich in these compounds followed by stem and then the roots.

The whole plant was noted with intermediate quantity. The results of antioxidant activity agree with the notion that these phytochemicals are the cause of antioxidant properties of the plant (Adedapo *et al*., 2009).

After 10 times dilution (at 0.1 mg /ml) the free radical scavenging for ascorbic acid noted was 44.9% (± 1.127). The whole plant demonstrated 27.43% (± 0.946) activity (next to leaves). The leaves showed a maximum (35.58% ± 1.202) inhibition, while roots the minimum (1.02% ± 0.909). Different dilutions had different impacts on DPPH scavenging activity. Comparing activity at 1mg/ml with 0.1 mg/ml (10 times dilution) shows a 53.84% decrease in ascorbic acid activity. At this dilution the activity of the whole plant reduced from 59.93% (± 1.058) to 27.43% (± 0.946), a 54.23% decrease. Roots from 55.96% (± 1.347) to 1.02% (± 0.909) with 98.2% decrease stem from 57.95% (± 1.924) to 11.67% (± 1.09) with 79.86% decrease and the leaves from 70.58% (± 0.195) to 35.58% (± 1.202) with a 49.58% reduction in activity. Decrease in activity of ascorbic acids (53.84%) in comparison to plant and plant parts might be explainable on the grounds of synergism. Ascorbic acid is a pure moiety and gradually decreased in concentration with dilution. On the other hand the plant extract is a mixture of a good number of compounds. On account of mix effect, researchers have noticed synergistic effects in plant (Kratchanova *et al*., 2010). As at high concentration there are more chances of synergism than at lower concentration therefore more activity was noted at high concentration than at low. More than a hundred disorders are reported to be the result of reactive free radicals (Pourmorad *et al*., 2006), while existing pathological conditions like diabetes mellitus be aggravated (Patel *et al*., 2010; Ahmed *et al*., 2008). The Present of good quantity of important phtochemicals and the high antioxidant potential support the medicinal use of the plant.

***Euphorbia prostrata* Aiton**

Percent inhibition of DPPH of whole plant, roots, stem and leaves are given and compared with ascorbic acid in Table 51.

Ten dilutions between 1 to 0.1 mg/ml were prepared each for the standard as well as for test samples. Ascorbic acid produced maximum inhibition of 98.59% (± 0.4) at 1mg/ml and 44.9% (± 1.127) at high dilution. *Euphorbia prostrata* also showed high

DPPH inhibition in leaves extract (73.07% ± 1.017), followed by stem (56.73% ± 1.017) and least in roots (48.91% ± 0.778). The whole plant showed optimum inhibition of 67.37% (± 0.949). This trend was present within all the nine dilutions.

When both these plants were compared it was seen that 1 mg/ml *E. prostrata* whole plant exhibited more DPPH inhibition (67.37 ± 0.949%) than *E. granulata* (59.93 ± 1.058%). Similar trend of high activity was also observed for leaves of *E. prostrata* (73.07 ± 1.017%). In case of roots, *E. granulata* exhibited more DPPH scavenging (55.96 ± 1.347%) than *E. prostrate* whole plant (48.91 ± 0.778%). Stems of both plants demonstrated identical inhibition of free radical. Similar pattern of activities for plant and plant parts were registered for all the dilutions. High free radical scavenging activity demonstrated by leaves, intermediate by whole plant, leaves and low activity shown by roots are in complete harmony with the phytochemical concentration in these parts (Tables 6, 7). Decrease in percent inhibition with dilutions was also different in both the plants. With 10 times dilution the activities of *E. prostrata* whole plant decreased by 60.0% compared to 50% decreased in *E. granulata*. In plant parts more decrease with dilution was noted for roots (90.17%), followed by stem (83.84%) and then leaves (64.47%). This trend was also observed in *E. granulata*. The different impacts on overall activities with dilution may be due to different combination of different plant chemicals.

I. Cytotoxic activity

Euphorbia granulata Forssk

Table 52 shows the results of the brine shrimp lethality bioassay. Table 53 summarizes the toxicity levels of plant extracts. The high toxic level determined was 25mg/ml where 97.5% naupli deaths recorded while the lower toxic level noted was 2 mg/ml with 100% survival. For this plant 16.21 was calculated as the LD50 (Table 52).

Out of 50 naupli only one death (2%) was recorded in negative control (DMSO). Similarly one death (2%) was also seen for 0.01 mg/ml extract concentration. There was 100% survival in 0.1 and 1.0 mg/ml doses. There is a direct correlation between toxicity of a therapeutic agent to brine shrimps nauplii and human nasopharyngeal carcinoma (Fatima *et al*., 2009). Cytotoxic property of natural products is also liked to its anticancer potential (Rumzhum *et al*., 2012; Habib *et al*., 2011)). For investigating anticancer

potential of natural products one or two anticancer activities are coupled and usually antitumor and cytotoxic activities follow each other (Hussain *et al*., 2007). In the present study standard concentrations of 10 µg, 100 µg and 1000 µg proposed by McLaughlin & Rogers (1998) were used for cytotoxic impact of the plant extracts. The results show that the ethanolic extracts were not lethal at all the three strengths (10 µg, 100 µg and 1000 µg). Similar results were achieved with negative control. However the positive control (P-dichromate) produced 100% lethality. This suggests that the extract was not toxic at standard concentration proposed by McLaughlin & Rogers (1998).

In a separate experiment toxic concentrations of the extract ranged from 1 to 40 mg/ml were determined. The least tolerable level (100% deaths) was noted with 25 mg/ml. The maximum concentration at which no death occurred was taken as highest tolerable level. Thus highest tolerable level determined was 2 mg/ml. After applying the correction factor for both high deaths and lowest death percentage the actual value come out to be 97.5% and 2.5%. Using these and probit values (Table 54), LD_{50} was determined. Plant extracts having $LD_{50} < 1$ mg are considered as toxic (Tatli *et al*., 2008). Using graphpad prism software 16.21 was determined as LD_{50} for the extract (Fig. 22). This suggests that the extract is not toxic for brine shrimps. Previously antitumor activity was carried out for *E. granulata* which showed no inhibition.

Hence the overall results suggest the non-cytotoxic nature of *Euphorbia granulata*. It further suggests that plant is safe for human consumption even if taken in higher doses.

***Euphorbia prostrata* Aiton**

The results of *Euphorbia prostrata* ethanolic extracts on brine shrimp larvae are given in Table 55. Toxicity study is presented in Table 56.

For negative control and 10 µg/ml of extract 98% survival was observed. No death occurred at 100 and 1000 µg/ml. Lower toxic level of 3 mg/ml and upper toxic level (97.5% deaths) of 30 mg/ml was observed for extract in the toxicity study (Table 56). LD_{50} calculated for *Euphorbia prostrata* was 18.62 (Table 55).

Comparing data for cytotoxic activity of *E. granulata* and *E. prostrata*, it was clear that at standard concentrations used (10, 100 and 1000 µg) both data sets coincide with each other. The only difference that can be detected with the two sets of data is the

difference in LD_{50} value. LD_{50} was determined using different concentrations of the plant extracts. *E. granulata* had LD_{50} of 16.21 while it was 18.62 in the case of *E. prostrata* (Figures 22, 23). The high LD_{50} indicates that higher concentrations of the *E. prostrata* are needed to produce same mortality as that of *E. granulata*. The identical results for the two plants also indicate that the two plants have almost identical chemical constituents. This might be one of the reasons why people use the two plants without distinction for different ailments.

J. Phytotoxic activity

Euphorbia granulata Forssk

1. Lemna bioassay technique

Inhibitory effects of *E. granulata* extract on the growth *Lemna minor* are presented in Table 57. The activity was also monitored on radish seeds germination and radical growth. The impacts are provided in Tables 59 and 60. Table 58 summarized the results of determining LC_{50} while Fig. 1 shows the log of dose versus probit values. Table 54 contains standard probit values for estimating LC_{50}.

Lemna bioassay: For this experiment the positive standard was glyphosate while the negative was DMSO. The results reveal that all the fronds survived in negative control while 100% inhibition was noted for positive control. At 0.01 mg/ml of extracts, no inhibition occurred while at 0.1 mg/ml the number of fronds increased from 45 to 57. Only one frond wilted (2%) at 1.0 mg/ml concentration. In self-defense plants produces certain compounds which directly inhibit growth of other plants. They may create repulsive smell and taste to protect from herbivores. These compounds are not always toxic but may be neutral or stimulatory for other organisms and are called allelochemicals (Inderjit & Callaway. 2003; Yasmin *et al*., 2011). Many biological molecules are high profile drugs. Natural products that show inhibitory effects on tumor growth have also shown inhibitory effect on lemna growth. Therefore, lemna bioassay is considered as one of the initial screening test for searching antitumor drugs (Hussain *et al*., 2010; Ayatollahi *et al*., 2010; Ahmad *et al*., 2011). The present study revealed no inhibitory effect at 0.01 and 1.0 mg/ml on the growth of *Lemna minor*. This was apparently a neutral impact. However, at 0.1 mg/ml concentration the number of lemna fronds

increase from 45 to 57 showing a significant increase (+27%) in the number of fronds. This indicates that the extract had stimulatory effect at specific dose. The toxic levels and LC_{50} were also determined (Fig. 24). At 5mg/ml strength no frond growth showed inhibition while the 100% inhibition was noted at 55 mg/ml. The high toxicity value of 55mg/ml and smallest safe value of 5 mg/ml led to high LC_{50} value. These values suggest that the plant is not harmful at ordinary dose and has no activity against weeds. Previously, the plant was checked for antitumor potential and the present results show a complete correlation in two activities. In case of antitumor activity stimulatory effect was noted for *Agrobacterium tumefaciens* by slightly increasing the number of produced tumors. Terpenes and phenols are considered as one of the phytochemicals that are produced as allelochemicals (Sarkar *et al*., 2012). In preliminary detection tests, terpenes and perpenoids were not detected in whole plant. This shows an agreement between the analytical portion and present activity. Growth hormones like auxin and cytokinin are important growth hormones. The present stimulant effect of extract might also be due to some compounds having growth hormones like effect (Shahid-ud-daula & Basher, 2009).

2. Radish seeds germination and growth method

The effect of extracts on seeds germination and radical elongation of radish are given in Tables 59 and 60, respectively.

Effect on seeds germination: At 7.5 mg/ml extract concentration, out of 150 seeds 141 germinated showing 94% germination. At 1.0 mg/ml 143 seeds germinated (95%). In negative control 139 seeds germinated (93%). Percent inhibition calculated at 7.5 mg/ml was 6% while at 1.0 mg/ml it was 4.7%. When compared to negative control the inhibition noted was 7.3%.

The standard criteria use for percent inhibition is, 0 to 39% represent low or poor activity, 40 to 59% moderate activity, 60 to 69% is good and above 70% is considered as significant activity (Ayatollahi *et al*., 2010)**.** Comparing with these standards, it appeared that the extract had no inhibitory effect against radish seeds germination. Studies shows that monoterpenoids and sesquiterpenoids plays important role in inhibiting the growth of seedlings by inhibiting cell division and inducing structural breaks in roots (Mancini *et al*., 2011). Our results suggest that *E. granulata* lacks these allelopathic compounds.

Effect on seeds radical growth: At 10 mg/ml of extract the roots mean length was 1.4 cm (± 0.41) on day one that increased in length to 5.5 cm (± 0.892) on day 5. Similar the mean root length recorded for 1.0 mg/ml on day 1 was 1.8 cm (± 0.262) which reached its maximum length of 5.4 cm (± 0.448) on last day of experiment. In negative control the growth recorded was 1.3 (± 0.401) on day 1 which attained 5.1 cm length (± 1.16).

The mean growth in all the three sets was almost equal that suggests no impact on overall growth. However, comparing the increases in length from day onward gives some indication of impact. The mean length of radical on day 1for 10 mg/ml strength was 1.4 cm. From this day onward the average increases in lengths were 293%. The root length for 1.0 mg/ml from 1.8 cm on day 1 to 5.4 cm on day 5 was a 200% increase in the mean roots length. Furthermore, the length of radical in negative control increased from 1.3 to 5.1 cm, a 292% increase. Carefully looking at these results shows that the increases in roots length for 10 mg and negative control are similar. This suggests two possibilities: In the first case the 1 mg dose looks inhibitory when compared to negative control. However, comparing the results of 1.0 mg with 10 mg doses, a stimulatory effect was observed with increase dose of extract. A final decision on these results if difficult therefore more evaluation is needed at many different doses for a clear allelopathic effect of plant.

It is concluded that *E. granulata* has no inhibitory effect on seed germination and seedling growth at all doses. However at 0.1 mg/ml dose the extracts enhanced the lemna growth. No inhibition of radish seeds germination was noted while the effect on radical elongation was ambiguous. Further study should be carried for identification of stimulatory compounds. While different doses study is needed for radish seeds radical elongation.

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Table 61 shows the effects of different doses of *E. prostrata* extract on *Lemna minor* growth. Table 62 shows LC_{50} determination values.

Lemna bioassay: The mortality was 100 % in positive control while there was 100% survival in negative control. The survival rate in 0.01 and 0.1 mg plant extract was

also 100%. One frond died in 1.0 mg/ml extract (98% survival). Compared to *E. granulata*, it was noted that 0.1 mg/ml dose of *E. granulata* had a stimulant effect while that effect was not found in *E. prostrata*.

In investigating the LC_{50} value, least mortality of 0% was recorded at 8 mg while high mortality of 100% occurred at 40 mg/ml. The resultant LC_{50} was 25.7 (Fig. 25). The graphic presentation of LC_{50} is presented in figure 2. The LC_{50} noted for *E. granulata* was 33.9 mg/ml while in present case it was 25.7. This 24.2% low LC_{50} for the present study reveals that *E. prostrata* is less toxic to lemna minor compared to *E. granulata*. Overall the present plant is not inhibitory to the growth of lemna at standard comcentrations of 1.0, 0.1 and 0.001 mg/ml. However, at higher concentrations the plant shows toxicity to *Lemna minor* but that toxicity was not as high as noted with *E. granulata*.

Effects on seeds germination: The germination was 98% at 7.5 mg, 91% at 1.0 mg/ml and 95.3% in negative control (Table 63). The results for *E. prostrata* suggest a complete correlation with that of *E. granulata*. In both plants more than 90% germination was noted for positive and negative control. Comparing this with the standard inhibition potential of the extract, the present inhibition was in poor activity range (Ayatollahi *et al*., 2010)**.** Hence the plant seems to have no allelopathic constituents for suppressing the germination of radish seeds.

Radish seeds radical growth: At day 1 the radical length at 10 mg was 0.8 that attained a maximum size of 4.2 cm on day 5 (Table 64). The range of length for 1 mg concentration was between 1.2 and 4.5 cm while it was between 1 and 4.5 for negative control. As the end results shows a length of 4.2, 4.5 and 4.5 for 10 mg, 1.0 mg and negative control. Looking at these lengthening effects on radical the results seems unclear and ambiguous. But comparing the percentage growth of radical length from day 1 to day 5, 425% increase was noted in radical length at 10mg dose, 275% increase at 1.0 mg dose and 350% for negative control. Further comparison of 1.0 mg/ml dose with negative control indicates as if the extracts have slowed down the lengthening activity of radical. However when compared 1.0 mg with 10 mg/ml doses an enhancement in lengthening

with increased dose is observed. These suggest that at low dose the extract is inhibitory to the lengthening activity of radical growth while at higher dose it seems stimulatory.

It is gained that *E. prostrata* is not inhibitory to *Lemna minor* growth and to germination of radish seeds. Radical growth was inhibited even at small doses. However the action was stimulatory at higher doses.

K. Insecticidal activity

Euphorbia granulata Forssk

Residual film toxicity: Extensive use of insecticidal agents resulted in developing resistance to the existing insecticides. Cross resistance and multi-resistance strains have also been reported (Silva *et al*., 2008; Vinayaka *et al*., 2010; Nikkon *et al*., 2009). The search for a natural agent that may have acceptable smell, environmental friendly, biodegradable and selective for predators' insects only is needed (Alam *et al*., 2009; Silva *et al*., 2008; Hong *et al*., 2006). Results for *E. granulata* with ten different dilutions of extracts against *Tribolium castaneum* are shown in Table 65. A 100% mortality was recorded with lindane while the negative control resulted in 6.6% mortality. The picture of mortality at different extract doses seems unclear. A 6.6% mortality was recorded at 10, 60, 90, 100 mg and negative control, 3.3% mortality at 20, 30, 80 mg and no mortality for 40 and 70 mg/ml. However, it was very clear that the total mortality resuling from different extract concentrations did not exceed 6.6%. Studies show a strong correlation between the medicinal plants and its pesticidal activity. Certain carbohydrates, phytosterols, saponins, tannins, flavonoids and phenols have larvicidal activity against mosquitos. Similarly tetracyclic phenols, prenylated xanthones, and saponins have activity against *A. aegypti* (Mallikarjun *et al*., 2010). Therefore *E. granulata* was tested against *Tribolium castaneum*. The results suggest no insecticidal effects against *Tribolium castaneum*.

Fumigant toxicity: Ten different concentrations of *E. prostrata* extract were used against 30 *Tribolium castaneum* for each concentration. Negative control was DMSO while positive control was lindane. The results are shown in Table 66. The mortality was 100% with lindane while there were no deaths with DMSO. Use of extract against insect revealed no deaths with 40 and 70 mg. with 20, 30, 80 mg / ml, 3.3% mortality occurred

while only 6.6% were noted with the rest of extract preparations. These results suggest weak or poor activity and are indicative of the absence of any active phyto-constituents. Therefore, the insecticidal role of *E. granulata* seems negative.

Repellency bioassay: in this study two concentrations of 50 and 100 mg/ml were applied. In each case 45 *Tribolium castaneum* were used and the movement between the two halves of the pertidishes were recorded. The results show that the insects were randomly distributed between the two halves without any preference for a particular side (Table 67). In % repellency column, the figures with positive values indicate repellency while those with negative values indicate attraction. During ten hours period of study, the insects spend its time either at negative control or at positive control. At 100 mg strength the insects showed high movements to positive control at 4, 5, 6, 7 and 10 hours while the rest of the time they moved toward the negative control. In a similar way, at 50 mg/ml strength most of the insects at 1, 3, 4, 7 and 10 hours were found present on positive pol and vice versa. This suggests that plants have no repellent smell for *Tribolium castaneum*.

The findings reveal that there was poor residual film toxicity, poor fumigant toxicity and repellency. No preference was shown for positive or negative control sides. Therefore the plant is not toxic to the insects.

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The results for both the species are compared.

Residual film toxicity: The results for residual film toxicity of *E. prostrata* are presented in Table 68. *E. prostrata* also showed poor activity against *Tribolium castaneum*. The highest mortality was 6.6% recorded for 30 and 70 mg/ml strengths. Furthermore, 3.3% inhibition occurred at 40, 50, 80, 90, 100 mg/ml and negative control while no deaths were recorded on 10, 20 and 60 mg/ml. Positive control produced 100% inhibition. These results had not shown any correlation of deaths with extract concentration. The present mortality seems not due to extract instead some other physiological events in insect physiology might have played the role. Silva *et al*, (2008) reported that although plant species of Euphorbiaceae Family have been tested for cytotoxic activity but not for insecticidal activity.

Fumigant toxicity: Table 69 shows the impact of *E. prostrata* extract on *Tribolium castaneum.* Thirty healthy insects were used. In all the cases only one dead insect was found in 10, 30, 70, 90 mg/ml and negative control which represent 3.3% mortality. A 6.7% mortality was recorded for 40 and 80 mg each. There was no mortality in 20, 50, 60 and 100 mg/ml treatment however there was 100% mortality in positive control. Looking at the concentration dependent killing in the two experiments it seems that both the extracts produced similar results. But when total mortality in the two experiments is compared a different picture comes, as discussed here. In the above setting a total of 300 tribulium insects were used for test samples and a total of 10 deaths were noted however in present case out of 300 insects only 8 killed insects were found. This shows 3.3% mortality in the previous experiment compared to 2.66% mortality in the present. Although the overall results suggests no mortality but if assumed, the extract of *E. granulata* is comparatively more toxic than that of *E. prostrata*.

Repellency bioassay: the movements of tribuliums between negative control and positive control each hour for ten hours is presented in Table 70. Positive values (+) indicate repellency while negative values (-) attraction. Table 70 shows that insects had no preference for positive or negative side' i.e. free movement between the two sides was observed. This suggests the plant smell was not repulsive for the insects and that there was no toxicity in the plant extract. These results are again in complete agreement with that of previous experiment for *E. granulata*.

Therefore, it can be concluded that both *E. granulata* and *E. prostrata* had no activity against the targeted insect.

L. Antibacterial activity

Euphorbia granulata Forssk

The minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) are given in Table 73. The results for the effect of extract on the growth inhibition of bacteria are presented in Table 71. The Combined effect of streptomycin and *E. granulata* extracts are presented in Table 71. Table 72 exhibits standard inhibition zones for streptomycin (the positive control).

Non rationale use of these antibiotics for the last few decades has evolved into a new concern of resistance (Nanasombat & Lohasupthawee, 2005; Johnson *et al.*, 2005). Bacteria are intelligently evolving and using different strategies to evade and combat the adverse effects of antibiotics. Some have developed resistance to one while other has developed resistance to many antibiotics. These are called multidrug resistant strain (MDS). Methacillin resistant strain of *Staphylococcus aureus* (MRSA) are of especial concern (Aliero & Afolayan, 2006). To avoid the evolution of super bacteria researchers are looking for super molecules from different sources including plants, animals and microbes. (Camacho-Corona *et al*., 2009; Kotan *et al*., 2007; Aboaba *et al.*, 2006; Babu & Subhasree, 2009). For plant extract, the high MICs values were recorded for *Enterococcus faecalis* and *Pseudomonas aeruginosa* (20 mg/ml each) while the low was recorded for *Serratia marcscens* (5 mg/ml). Based on these MIC ranges test doses were set as 5, 10, 20, and 40 mg/ml (Cutler & Wilson, 2004). Eight bacterial species, including five Gram-negative and three Gram-positive were tested for sensitivity. The sensitivity results for positive control shows that six bacterial species were susceptible with zone of inhibition more than 15mm (Table 71). Two species' i.e. *Enterococcus faecalis* and *Klebsiella pneumonia* had intermediate sensitivity with inhibition zone between 12 to 14mm (Table 72). Compared to negative control of 6.0 mm (± 0.167) inhibition zone, the 40 mg/ml dose was effective against *Klebsiella pneumonia* with 8.6 mm (± 0.033) zones. The inhibition zone produced with reference drug against this bacterium was 13.1 mm (± 0.167). Furthermore, combined effect of plant extract with reference drug produced 15.7 mm (± 0.167) inhibition zone. This zone was 2.6 mm larger than the reference drug alone. This enlargement of zone in combination with plant extract suggests the additive property of the extract. The least sensitive species to 40 mg/ml dose was *Enterococcus faecalis* with 7.5 mm (± 0.058). However, the extract was found highly effective against *Serratia marcscens* that generated maximum of 9.5 mm (± 0.033) inhibition zone. For this bacterium, the positive control produced 21 mm (± 0.176) while in combination with extract 23.5 mm (± 0.088) hollow. The resultant zone was 2.5 mm more than reference zone (Table 71). All other bacterial species exhibited the same response to the plant extract.

Plants contain different phytochemicals which can be potential antibacterial (Sukanya *et al*., 2009). In the present study, the ethanolic extract of *Euphorbia granulata* was evaluated against eight human pathogenic bacteria. For synergism effect, combinations of reference drug with plant extracts were used. For all the eight bacterial species the maximum inhibition zone was recorded at 40mg/ml. At 5mg concentration the inhibition zone produced was not significant. The overall results show that with increasing concentration the activity against the bacterial strain the activity also increased. For example, the zone of inhibition at 5 mg concentration was 7.3mm (± 0.058) for *Klebsiella pneumonia,* while at 40mg/ml the zone was 8.6 (± 0.033). This indicated that the plant extract had some active ingredients for inhibiting bacterial growth. Table 71 showed that inhibition zone smaller than 11mm indicate resistance but these are reference zones for reference drugs however the extract is in crude form. Compare to standard drug and standard zone sizes, the inhibition zone obtained was small but possibly in crude extract the active metabolite may be in extremely small concentration (Ahmed & Abdelgaleil, 2005). The inhibition zone produced by combined effect of standard drug and extract also support the antibacterial potential. In combination the largest inhibition zone against *Serratia marcscens* was 23.5 (± 0.088). This zone is 2.5mm larger than the inhibition zone produced by streptomycin alone. The increase in size is almost equal to the effect of extract (8.6mm) plus streptomycin. Similar additive effects were also recorded for other bacterial species as well. The available antibiotics inhibit bacterial growth by either interfering with cell wall synthesis, membrane permeability, nucleic acid or protein synthesis. Researchers have noticed that the antibacterial activity of most of the plants is due to phenolic compounds and its different forms present in the extract (Shan *et al*., 2007; Dorman & Deans, 2000). There is no clear known mechanism for their antibacterial action. Some possible actions include destruction of membrane bound protein and ezymes, disruption of cell membrane, leakage and coagulation of cytoplasm. It is also believed that these compounds interfere with electromotive forces, DNA and RNA synthesis, degrade mitochondria and destroy proteins. These mechanisms vary with type of microorganism and with change in structure of cell wall, cell membrane and organelles in cytoplasm (Shan *et al*., 2007).

Prior to antibacterial activity *E. prostrata* was analyzed for phenolic compounds, flavonoids, alkaloids, tannins, glycosides and saponins, and were found with good quantity. The present antibacterial effect might be due to the presence and structural diversity of these compounds.

It can be concluded that *E granulata* had the potential of inhibiting bacterial growth at high concentration.

***Euphorbia prostrata* Aiton**

Table 75 shows the MIC and MBC values obtained for *Euphorbia prostrata*. Table 74 depicts the inhibition zones produced with reference drug, plant extract, negative control (DMSO) and combined effects of extract and standard drug.

Lowest MIC was noted for *Serratia marcscens* (5mg/ml) while highest for *Enterococcus faecalis* and *Pseudomonas aeruginosa* (20 mg/ml each). As the minimum and maximum MIC was similar to the previous study, the extracts were tested in the same concentration as used for *E. granulata* (Cutler & Wilson, 2004).

The appearance of resistance bacterial species to the existing therapeutic regime has posed a threat to the human health. Plants with a good diversity of secondary metabolites seem to be possible alternative to overcome the resistance problem (Roopashree *et al*., 2008). In present study the ethanolic extract of *E. prostrata* was used. Again, the most effective dose was 40 mg/ml. At this concentration, the most sensitive bacterial species was *Serratia marcscens*. The maximum inhibition zone formed by plant extract was 9.0 mm (± 0.067). The hollow produced with combined effect of drug and extract was 22.5 ± 0.088 for the most sensitive species. Compared to zone produced by standard drug (21 ± 0.176), this was 1.5 mm larger zone. Although, the contribution by the extract to the zone sizes was small but this is indication of additive effect. Three of the test species' i.e. *Klebsiella pneumonia, Citrobacter frundii* and *Enterococcus faecalis* were recorded with 7.5 mm inhibition zone when treated with 40 mg/ml of extracts. *Bacillus cerus, Streptococcus aureus, Pseudomonas aeruginosa* and *Escheritia coli* showed intermediate sensitivity with 7.5 and 9.0 mm inhibition zone. The additive property of extract was detected against all eight bacterial strains (Table 74). As these

results were obtained with crude extract, there is possibility that isolated active constituents may be potent antibacterial moiety (Ahmed & Abdelgaleil, 2005). Researchers links the antibacterial potential of plants extract to the presence of phenolic compounds (Shan *et al*., 2007; Dorman & Deans. 2000). This plant was analyzed for six phytochemicals i.e. total phenolics, flavonoids, alkaloids, tannins, glycosides and saponins and was found rich (Tables 6 & 7). The presence of these compounds might have contributed to the antibacterial effect of *Euphorbia prostrata*.

It is concluded that *E. prostrata* had some active principals that inhibit the growth of bacteria. Both *E. granulata* and *E. prostrata* have almost three time higher MBC. This means that the extract kills bacteria only at higher concentrations. This indicates that the extract is bacteriostatic in action.

M. Antifungal activity

***Euphorbia granulata* Forssk**

Ethanolic extracts of *E. granulata* were tested for antifungal activity. Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were determined (Table 78). The results of inhibition zone for different concentrations of extract against different fungal strains are summarized in Table 76. Table 77 shows the standard sizes for negative, weak, moderate and strong antifungal effects. To check for the interaction between plant extract and positive control, combined effect of the two was also studied (Table 76).

Four fungal strains; *Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus* and *Fusarium oxysporum* were used. The effective concentration for inhibiting growth of all tested strains (MIC) was 10 mg/ml. The minimum fungicidal concentration determined was 40 mg/ml. based on these observations 5, 10, 20 and 40 mg/ml strengths were used to check the sensitivity of these strains. DMSO was used as negative control while fluconazole as positive control. The zone of inhibition noted for negative control was 6.0 mm for all strains.

Fungi have some unique features like cell wall structure, cytoplasmic organells, lack of peptidoglycan and chlorophylls and ergosterol. These features both differentiate

and make its resemblance with bacteria, plants and animals (Ryan & Ray, 2004; Betsy & Keogh, 2005). Treating fungal infection is a challenging task both for scientists and physicians. Similarities between fungi and of host cell structure make the therapeutic agent to target functionality of the host cell also (Prescott *et al*., 2002). To avoid toxicity of these synthetic molecules the search for a safe solution can only be with plant or other living organisms (Ahmed & AbdelGaleil, 2005; Bi *et al*., 2008; Shin & Kang, 2002). In the present study the plant extract showed different activities at different concentrations and different activities against different strains. Compared to negative and positive controls, 5 mg/ml had no activity against *Aspergillus niger* (inhibition zone 6.0 ± 0.115). At this concentration weak activity was noted for other three strains. Out of four concentrations the most effective was 40 mg/ml. *Aspergillus niger* was recorded as comparatively more sensitive to 40 mg extract doses with zone size of (8.6 ± 0.115). This indicates that *Aspergillus niger* was resistant to the extract effect at low doses but most sensitive at high extract doses compared to other fungal strains. At high effective dose the second most sensitive strain was *Fusarium oxysporum* with inhibition zone of 8.3 mm (± 0.1). *Aspergillus flavus, Aspergillus niger* and *Aspergillus parasiticus* had intermediate sensitivity ranged between 7.8 mm (± 0.1) and (8.0 ± 0.2) zone. Furthermore, careful observation reveals that fluconazole combined with extract produced hollow of larger size than the drug alone. With fluconazole alone, the inhibition zone was 13mm (± 0.2) but in combination with extract the zone size enlarged to 15mm (0.115) for *Aspergillus niger*. There was an increase of 2mm with addition of plant extract to standard drug. The 2mm increase in zone size is definitely contributed by the plant extract. The increase in zone size was also noted for the rest of the fungal strains. This combined effect of plant extract and fluconazole suggests the antifungal potential of the plant. Antifungal drugs interfere with ergosterol synthesis, inhibit fungal enzyme Cytochrome P-450 or prevent mitosis by inhibiting spindle formation during cell division (Cowan, 2012; Kavanagh, 2011; Betsy & Keogh, 2005). Researchers contributes the antimicrobial role of plant extracts to phytochemicals like essential oil, phenolics, polyphenolics, alkaloids, terpenoids, polypeptides and others (Bansod & Rai. 2008; Bokhari, 2009; Rivillas-Acevedo & Garcia, 2007). How these compounds arrest microbial growth is poorly

understood but it is believed that different substitutions at different places in the phenolic structure influence the enzymes activity, proteins translocation and electron transport in microbilal cells (Dorman & Deans, 2000). Table 6 and 7 revealed reasonable quantities of total phenolics, flavonoids, alkaloids, tannins, glycosides and saponins *in E. granulata.* These phytochemicals could have arrested the fungal growth in present study.

It can be concluded that *E. granulata* exhibited antifungal properties against the four tested strains.

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The activities of *E. prostrata* extract against selected fungal strains are presented in Table 70. The MIC and MFC values are provided in Table 80.

The MIC value recorded for *Aspergillus flavus* was 5 mg/ml while the growth of *Aspergillus niger, Aspergillus parasiticus* and *Fusarium oxysporum* was inhibited at 10mg/ml each. The minimum MFC value recorded was 30mg / ml for *Aspergillus flavus while* the highest value was 50mg/ml for *Aspergillus niger* and *Aspergillus parasiticus*. Based on these values, 5, 10, 20 and 40mg/ml dilutions were used to determined sensitivities of fungal strains to plant extracts. Compared to negative control, 40mg/ml was found more effective for inhibiting fungal growth. *Aspergillus flavus* was noted as the most sensitive fungal species at this dose. Large inhibition zone of 9.0 mm (±0.115) was produced against *Aspergillus flavus*. The least sensitive strain to plant extract was *Aspergillus parasiticus*. For this species, 5 mg/ml had no activity while at 40 mg; a zone 7.5mm (±0.1) was recorded.

Additive property was also noted for *E. prostrata* against all fungal strains (Table 79). A 20 mm (± 0.289) inhibition zone was produced against *Aspergillus flavus* with fluconazole. Combining fluconazole with plant extract produced 22.5 mm (± 0.289), which is 2.5 mm larger than zone of fluconazole alone. The inhibition of fungal growth with plant extract and the additive property of extract suggest that the extract is effective against fungi especially *Aspergillus flavus*. Although, the inhibition hollow produced with extracts looks insignificant when compared to Table 77. Researchers have mentioned that plant extract contain active principal in minute amount therefore produce

small effects (Ahmed & Abdelgaleil, 2005). The present of different metabolites mentioned may have contributed to the present acitivity.

It is concluded that ethanolic plant extracts from both *E. granulata* and *E. prostrata* are potential inhibitors to microbial growth.

N. Antitumor activity

Euphorbia granulata Forssk

The results for tumor suppressing potential of *E. granulat* on potato discs are shown in Table 81while Table 82 shows the results on carrot discs.

Agrobacterium tumefaciens is a gram negative bacterium usually found in soil throughout the world. The bacteria induces tumor near the junction of roots and stem in both angiosperms and gymnosperms (Islam *et al*., 2010; Rathnavijaya *et al*., 2011). The bacteria produces tumor in experimentally designed potato discs and this is a well-known proof that the bacteria have tumor inducing capability.

In present study for negative control potato discs, 20 (±1.851) tumors were recorded. However, no growth was found on the discs treated with 3.125 µg of vincristine. The discs applied with 1mg and 0.5 mg of extracts developed 22 growths. Repeating the procedure on carrot discs resulted in 15 tumors for negative control and none for positive control. There were 14 tumors produced each for discs applied with 1.0, 0.5, 0.25mgs of extracts. The inhibition was 6.67%. Discs applied with 0.125 and 0.062 had 15 tumors each.

For measuring LC50, some discs were applied with high doses of extract. The LC_{50} determined for *Euphorbia granulata* was 15mg of extract per disc.

A 20% tumor inhibition suggested the positive activity (Ateeq-ur-Rehman *et al*., 2009). Tumor inhibition percentage on potato discs was only 5% at 62.5 µg/disc which reduces to (-5%) at 125 µg and to (-10%) at 0.5 and 1.0 mg/disc. This effect does not seem inhibitory rather looks stimulatory. It is said that the Ti-plasmid is responsible for the production of growth hormones including auxin and cytokinin. This increase cell division and which in turn leads to the formation of abnormally grown structure called as crown gall (Islam *et al*., 2010). The enhanced growth with plant extract may possibly

be providing growth hormones or stimulate production of growth hormones. The effect may also be growth stimulation of bacterium or increasing efficiency of plasmid insertion. Insignificant inhibitory effect of extract was noted on carrot discs. This was 6.67% inhibition and was noted with high extract concentration (1.0mg/disc). The IC_{50} was determined and found 25mg/disc. This high concentration looks toxic concentration for bacterium rather than inhibitory for tumor. The overall results show that the extract in not inhibitory for tumor rather it shows slight (10%) stimulatory effect on potato discs.

***Euphorbia prostrata* Aiton**

Percent inhibition of tumor growth with plant extracts on potato discs and on carrots discs are given in Table 83 and 84 respectively.

No inhibition or stimulation was noted on potato discs applied with 62.5 and 125µg of extracts (Table 83). While 15.79% inhibition was observed with 1.0 and 0.5mg of extracts.

The effects on carrot discs showed same neutral response at 62.5 and 125µg of extracts. However, the number of tumors increased by 27.27% with 1.0 mg and 9.09% with each 0.5mg and 0.25 mg of extracts compare to negative control. This increase in tumor number indicates a stimulant effect. The LC_{50} measured for *E. prostrata* was 20mg per disc.

The results obtained for *E. prostrata* were almost similar to *E. granulata*. No significant inhibitory effect was noted in the present extract or previous. Furthermore the high LC_{50} of 20mg/disc seems toxic effect of high concentration for bacterium than inhibitory effect for tumor. In case of *E. granulata* stimulatory effect was noted on potato discs but in present study the stimulatory effect was noted on carrot discs. Summing up revealed that the plant have no anti-tumor effect. However some increase in tumor number was noted.

Table 50. Diphenyl picrylhydrazyl (DPPH) scavenging potential of *E. granulata.*

Concentration mg/ml	Percent inhibition activity of *Euphorbia granulata* and its parts				
	Whole plant	Roots	Stem	Leaves	Ascorbic acid
1.0	59.93 ± 1.058	55.96 ± 1.347	57.95 ± 1.924	70.58 ± 0.195	98.59 ± 0.4
0.9	54.74 ± 1.158	50.96 ± 1.017	52.56 ± 1.109	67.18 ± 0.802	86.2 ± 2.118
0.8	51.02 ± 1.058	45.06 ± 0.907	41.35 ± 1.021	62.37 ± 1.237	79.5 ± 1.159
0.7	47.44 ± 0.29	33.01 ± 1.233	34.75 ± 1.392	58.01 ± 1.058	72.5 ± 0.192
0.6	42.11 ± 1.73	23.2 ± 1.058	30.9 ± 1.392	52.63 ± 1.31	67.37 ± 0.8
0.5	37.05 ± 0.949	16.15 ± 0.84	27.69 ± 1.501	47.11 ± 0.999	64.2 ± 1.346
0.4	32.31 ± 1.068	8.59 ± 1.125	21.41 ± 1.125	40.58 ± 1.017	58.4 ± 0.948
0.3	31.67 ± 0.949	5.76 ± 1.017	17.63 ± 1.061	38.65 ± 1.202	53.9 ± 0.949
0.2	30.25 ± 0.296	2.18 ± 1.125	14.36 ± 0.986	36.54 ± 0.881	48.0 ± 0.908
0.1	27. 43 ± 0.946	1.02 ± 0.909	11.67 ± 1.09	35.58 ± 1.202	44.9± 1.127
NC	0.0	0.0	0.0	0.0	0.0
Decrease in activity	49.58%	98.2%	79.86%	54.23%	53.84%

Values are mean of three analyses with ± SD.

Table 51. Diphenyl picrylhydrazyl (DPPH) scavenging potential of *E. prostrata.*

Concentration mg/ml	Percent inhibition activity of *Euphorbia prostrata* and its parts				
	Whole plant	Roots	Stem	Leaves	Ascorbic acid
1.0	67.37 ± 0.949	48.91 ± 0.778	56.73 ± 1.017	73.07 ± 1.017	98.59 ± 0.4
0.9	63.08 ± 0.195	41.22 ±1.389	50.83 ± 0.907	70.7 ± 0.296	86.2 ± 2.118
0.8	58.52 ± 1.392	32.95 ± 0.949	45.06 ± 0.907	67.56 ± 0.801	79.5 ± 1.159
0.7	53.78 ± 1.058	27.05 ± 1.096	40.64 ± 1.31	61.47 ± 1.092	72.5 ± 0.192
0.6	50.06 ± 0.801	23.27 ± 1.347	35.58 ± 0.881	53.9 ± 1.308	67.37 ± 0.8
0.5	45.13 ± 0.949	19.49 ± 1.308	31.09 ± 1.096	47.63 ± 0.29	64.2 ± 1.346
0.4	39.42 ± 1.167	16.47 ± 0.952	27.37 ± 0.949	41.15 ± 1.017	58.4 ± 0.948
0.3	35.51 ± 1.128	13.84 ± 1.202	20.13 ± 0.985	34.87 ± 0.62	53.9 ± 0.949
0.2	29.81 ± 1.021	9.87 ± 0.801	14.42 ± 0.882	31.54 ± 1.202	48.0 ± 0.908
0.1	27.63 ± 1.456	4.81 ± 0.881	9.17 ± 0.488	25.96 ± 0.808	44.9± 1.127
NC	0.0	0.0	0.0	0.0	0.0
Decrease in activity	60.0%	90.17%	83.84%	64.47%	53.84%

Values are the mean of three with ±SD

Table 52. Impact of *Euphorbia granulata* ethanolic extracts on brine shrimp larvae.

Parameters	NC	Extract concentration used			PC
	DMSO	0.01mg / ml	0.1 mg / ml	1.0 mg / ml	P-dichromate
No of nauplii used	50	50	50	50	50
Nauplii survived	49	49	50	50	0
Dead nauplii	1	1	0	0	50
Percent survival	98	98	100	100	0
LD_{50} (µg/ml)	-	16.21	16.21	16.21	-
Upper toxic concentration	-	25.0	25.0	25.0	-
Lower toxic concentration	-	2.0	2.0	2.0	-

The experiment was performed in five replicates for each dilution, NC = negative control, PC = positive control

Note. Log value against probit 5 was 1.21 and its antilog value was 16.21 (LD_{50})

Table 53. LD_{50} Calculations for *Euphorbia granulata* for brine shrimps larvae.

Groups	Dose (mg)	Log of dose	Total naupli	Dead naupli	Percent dead	Corrected percentage	Probit value
1	2.0	0.30	50	0	0	2.5	3.04
2	8.0	0.90	50	6	12	12	3.82
3	14.0	1.15	50	17	34	34	4.59
4	20.0	1.30	50	38	76	76	5.71
5	25.0	1.40	50	50	100	97.5	6.96

Table 54. Conversion table from percentage to probit value.

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Table 55. Impact of *Euphorbia prostrata* ethanolic extracts on brine shrimp larvae.

Parameters	NC	Extract concentration used			PC
	DMSO	10 µg/ml	100 µg/ml	1000 µg/ml	P-dichromate
No of nauplii used	50	50	50	50	50
Nauplii survived	49	49	50	50	0
Dead nauplii	1	1	0	0	50
Percent survival	98	98	100	100	0
LD_{50} (µg/ml)	-	18.62	18.62	18.62	-
Upper toxic concentration	-	30	30	30	-
Lower toxic concentration	-	3	3	3	-

The experiment was performed in five replicates for each dilution, NC = negative control, PC = positive control

Note. Log value against probit 5 was 1.27 and its antilog value was 18.62 (LD_{50})

Table 56. LD_{50} Calculations for *Euphorbia prostrata* for brine shrimps larvae.

Groups	Dose (mg)	Log of dose	Total naupli	Dead naupli	Percent dead	Corrected percentage	Probit value
1	3	0.48	50	0	0	2.5	3.04
2	8	0.90	50	4	8	8	3.59
3	13	1.11	50	13	26	26	4.36
4	21	1.32	50	29	58	58	5.20
5	30	1.47	50	50	100	97.5	6.96

Table 57. Effect of ethanolic extract of *Euphorbia granulata* on *Lemna minor* growth.

Parameters	NC	Extract concentration used			PC
	DMSO	0.01mg / ml	0.1 mg / ml	1.0 mg / ml	Glyphosate
No of fronds used	45	45	45	45	45
Fronds survived	45	45	57	44	0
Dead fronds	0	0	(- 12)	1	45
LC_{50} (mg / ml)	-	33.88	33.88	33.88	-
UTC (mg/ml)	-	55.0	55.0	55.0	-
LTC (mg/ml)	-	5.0	5.0	5.0	-

NC = negative control, PC = positive control, UTC = upper toxic concentration, LTC = lower toxic concentration,

The experiment was performed in three replicates for each dilution. The negative values represent the increase in fronds number.

Table 58. Calculating LC_{50} of *Euphorbia granulata* for *Lemna minor*.

Groups	Dose (mg)	Log of dose	Total fronds	Dead fronds	Percent dead	Corrected percentage	Probit value
1	5.0	0.70	45	0	0	2.5	3.04
2	17.0	1.23	45	3	6.7	6.7	3.49
3	29.0	1.46	45	17	37.8	37.8	4.68
4	41.0	1.61	45	34	75.5	75.5	5.69
5	55.0	1.74	45	45	100	97.5	6.96

Note. Log value against probit 5 was 1.53 and its antilog value was 33.88 (LD_{50})

Table 59. Impact of *E. granulata* extract on radish seeds germination.

Concentration mg/ml	Seeds used	Seeds germinated	Percent germination
7.5	150	141	94
1.0	150	143	95.3
Negative control	150	139	92.7

Table 60. Impact of *Euphorbia granulata* extract on radical elongation of radish seeds.

Concentration mg/ml	Radical length with ±SD				
	D1	D2	D3	D4	D5
10.0	1.4 ± 0.41	2.6 ± 0.576	3.5 ± 0.656	4.1 ± 0.719	5.5 ± 0.892
1.0	1.8 ± 0.262	2.4 ± 0.251	3.7 ± 0.428	4.7 ± 0.405	5.4 ± 0.448
Negative control	1.3 ± 0.401	2.1 ± 0.558	3.4 ± 0.853	4.3 ± 1.023	5.1 ± 1.16

Table 61. Effect of ethanolic extract of *Euphorbia granulata* on *Lemna minor* growth.

Parameters	NC	Extract concentration used			PC
	DMSO	0.01mg/ml	0.1 mg/ml	1.0 mg/ml	Glyphosate
No of fronds used	45	45	45	45	45
Fronds survived	45	45	45	44	0
Dead fronds	0	0	0	1	45
LC_{50} (mg / ml)	-	25.7	25.7	25.7	-
UTC (mg/ml)	-	40.0	40.0	40.0	-
LTC (mg/ml)	-	8.0	8.0	8.0	-

The values are in cms. The experiment was performed in three replicates for each dilution NC = negative control, PC = positive control, UTC = Upper toxic concentration, LTC = Lower toxic concentration,

Table 62. Calculating LC_{50} of *Euphorbia granulata* for *Lemna minor*.

Groups	Dose (mg)	Log of dose	Total fronds	Dead fronds	Percent dead	Corrected percentage	Probit value
1	8.0	0.90	45	0	0	2.5	3.04
2	16.0	1.20	45	7	15.5	15.5	3.98
3	24.0	1.38	45	20	44.5	44.5	4.86
4	32.0	1.50	45	30	66.6	66.6	5.42
5	40.0	1.60	45	45	100	97.5	6.96

Note. Log value against probit 5 was 1.41 and its antilog value was 25.7 (LD50)

Table 63. Impact of *Euphorbia prostrata* extract on radish seeds germination.

Concentration mg/ml	Seeds used	Seeds germinated	Percent germination
7.5	150	147	98
1.0	150	137	91.3
Negative control	150	143	95.3

Table 64. Impact of *Euphorbia prostrata* extract on radical elongation of radish seeds.

Concentration mg/ml	Radical length with ±SD				
	D1	D2	D3	D4	D5
10.0	0.8 ± 0.334	1.4 ± 0.458	2.6 ± 0.689	3.4 ± 0.868	4.2 ± 1.011
1.0	1.2 ± 0.436	2.0 ± 0.535	3.1 ± 0.763	3.8 ± 0.915	4.5 ± 1.113
Negative control	1.0 ± 0.301	1.7 ± 0.451	2.5 ± 0.626	3.5 ± 0.831	4.5 ± 1.042

Table 65. 24 hours residual film toxicity effects of *E. granulata* on *Tribolium castaneum.*

Concentration (mg/ml)	No of Insects	Dead insects	Insect survived	% Mortality
10	30	2	28	6.6
20	30	1	29	3.3
30	30	1	29	3.3
40	30	0	30	0
50	30	3	27	10
60	30	2	28	6.6
70	30	0	30	0
80	30	1	29	3.3
90	30	2	28	6.6
100	30	2	28	6.6
Positive control	30	30	0	100
Negative control	30	2	28	6.6

Table 66. 24 hours fumigant toxicity effects of *E. granulata* on *Tribolium castaneum.*

Concentration (mg/ml)	No of Insects	Dead insects	Insect survived	% Mortality
10	30	1	29	3.3
20	30	1	29	3.3
30	30	0	30	0
40	30	1	29	3.3
50	30	2	28	6.6
60	30	0	30	0
70	30	2	28	6.6
80	30	1	29	3.3
90	30	0	30	0
100	30	2	28	6.6
Positive control	30	30	0	100
Negative control	30	0	30	0

Table 67. 10 hours repellency bioassay effects of *E. granulata* on *Tribolium castaneum.*

Time interval	Effects of the two concentrations					
	100 mg/ml			50 mg/ml		
	PIN	PIP	% Repellency	PIN	PIP	% Repellency
1 hour	57.8	42.2	15.6	48.9	51.1	-2.2
2 hour	51.1	48.9	2.2	57.8	42.2	15.6
3 hour	53.3	46.7	6.6	44.4	45.6	-11.2
4 hour	48.9	51.1	-2.2	42.2	57.8	-15.6
5 hour	48.9	51.1	-2.2	51.1	48.9	2.2
6 hour	44.4	45.6	-11.2	51.1	48.9	2.2
7 hour	42.2	57.8	-15.6	45.6	44.4	-8.8
8 hour	51.1	48.9	2.2	51.1	48.9	2.2
9 hour	51.1	48.9	2.2	53.3	46.7	6.6
10 hour	45.6	44.4	-8.8	48.9	51.1	-2.2

PIN = percentage of insect on negative control, PIP = percentage of insect on positive control

Positive values (+) indicated repellency and negative values (-) attraction.

Table 68. 24 hours residual film toxicity effects of *E. prostrata* on *Tribolium castaneum.*

Concentration (mg/ml)	No of Insects	Dead insects	Insect survived	% Mortality
10	30	0	30	0
20	30	0	30	0
30	30	2	28	6.6
40	30	1	29	3.3
50	30	1	29	3.3
60	30	0	30	0
70	30	2	28	6.6
80	30	1	29	3.3
90	30	1	29	3.3
100	30	1	29	3.3
Positive control	30	30	0	100
Negative control	30	1	29	3.3

Table 69. 24 hours fumigant toxicity effects of *E. prostrata* on *Tribolium castaneum.*

Concentration (mg/ml)	No of Insects	Dead insects	Insect survived	% Mortality
10	30	1	29	3.3
20	30	0	30	0
30	30	1	29	3.3
40	30	2	28	6.6
50	30	0	30	0
60	30	0	30	0
70	30	1	29	3.3
80	30	2	28	6.6
90	30	1	29	3.3
100	30	0	30	0
Positive control	30	30	0	100
Negative control	30	1	29	3.3

Table 70. 10 hours repellency bioassay effects of *E. prostrata* on *Tribolium castaneum.*

Time interval	Effects of the two concentrations					
	100 mg/ml			50 mg/ml		
	PIN	PIP	% Repellency	PIN	PIP	% Repellency
1 hour	44.4	45.6	-11.2	48.9	51.1	-2.2
2 hour	57.8	42.2	15.6	51.1	48.9	2.2
3 hour	48.9	51.1	-2.2	51.1	48.9	2.2
4 hour	42.2	57.8	-15.6	51.1	48.9	2.2
5 hour	48.9	51.1	-2.2	53.3	46.7	6.6
6 hour	51.1	48.9	2.2	45.6	44.4	-8.8
7 hour	51.1	48.9	2.2	48.9	51.1	-2.2
8 hour	51.1	48.9	2.2	42.2	57.8	-15.6
9 hour	53.3	46.7	6.6	44.4	45.6	-11.2
10 hour	45.6	44.4	-8.8	57.8	42.2	15.6

PIN = percentage of insect on negative control, PIP = percentage of insect on positive control

Table 71. Inhibitory effect of *E. granulata* extract against ATCC bacterial strains.

	Inhibition zones (mm)						
	Plant Extracts				Controls (+ ive and – ive)		
Bacterial Species	5mg	10mg	20mg	40mg	NC	10µg Strp.	P.Ex.+ Strp.
Klebsiella pneumonia 700603ATCC	7.3 ± 0.058	7.3 ± 0.033	7.5 ± 0.033	8.6 ± 0.033	6.0 ± 0.167	13.1 ± 0.167	15.7 ± 0.167
Bacillus cerus QUI-ATCC	7.2 ± 0.033	7.2 ± 0.033	7.9 ± 0.033	8.5 ± 0.033	6.0 ± 0.033	15.3 ± 0.145	19.1 ± 0.088
Citrobacter frundii QUF-ATCC	6.0 ± 0.0	6.5 ± 0.033	7.0 ± 0.0	7.5 ± 0.033	6.0 ± 0.0	11.5 ± 0.0	12.5 ± 0.033
Streptococcus aureus 25923ATCC	7.0 ± 0.1	7.4 ± 0.033	8.0 ± 0.033	8.0 ± 0.067	6.0 ± 0.067	18 ± 0.167	20.3 ± 0.167
Enterococcus faecalis 29212ATCC	6.3 ± 0.033	6.3 ± 0.033	7.0 ± 0.067	7.5 ± 0.058	6.0 ± 0.0	13 ± 0.1	14.8 ± 0.033
Pseudomonas aeruginosa 27853ATCC	6.5 ± 0.033	6.8 ± 0.033	7.0 ± 0.033	7.5 ± 0.058	6.0 ± 0.0	20 ± 0.1	21.6 ± 0.1
Escheritia coli 25922ATCC	7.0 ± 0.0	7.5 ± 0.033	7.8 ± 0.033	8.5 ± 0.285	6.0 ± 0.0	19 ± 0.26	21.5 ± 0.067
Serratia marcscens (strain from local water)	8.5 ± 0.033	8. 5 ± 0.0	8.8 ± 0.033	9.5 ± 0.033	6.0 ± 0.0	21 ± 0.176	23.5 ± 0.088

Note. zone size includes 6mm well size and the result is mean of 3 values with ± SEM.

P.Ex. = Plant extract, Strp. = Streptomycin, NC= negative control (DMSO)

Table 72. Standard Inhibition zone in for 10 µg / well streptomycin (Prescott, 2002).

Zone size	Results
≤ 11 mm	Resistant
12 – 14 mm	Intermediate
≥ 15 mm	Susceptible

Table 73. MIC and MBC investigated for *E. granulata* in different bacterial species.

Bacterial Species	MIC (mg/ml)	MBC (mg/ml)
Klebsiella pneumonia 700603ATCC	15	40
Bacillus cerus QUI-ATCC	10	25
Citrobacter frundii QUF-ATCC	10	30
Streptococcus aureus 25923ATCC	15	50
Enterococcus faecalis 29212ATCC	20	60
Pseudomonas aeruginosa 27853ATCC	20	60
Escheritia coli 25922ATCC	15	50
Serratia marcscens (strain from local water)	5	20

Table 74. Inhibitory effect of *E. prostrata* extract against ATCC bacterial strains.

	Inhibition zones (mm)						
	Plant Extracts				Controls (+ ive and – ive)		
Bacterial Species	10mg	20mg	30mg	40mg	NC	10µg Strp.	P.Ex.+ Strp.
Klebsiella pneumonia 700603ATCC	6.5 ± 0.033	6.8 ± 0.033	7.0 ± 0.033	7.5 ± 0.058	6.0 ± 0.0	13.1 ± 0.167	15.5 ± 0.067
Bacillus cerus QUI-ATCC	7.0 ± 0.68	7.0 ± 0.033	7.5 ± 0.0	8.0 ± 0.058	6.0 ± 0.0	15.3 ± 0.145	16.5 ± 0.088
Citrobacter frundii QUF-ATCC	6.0 ± 0.0	6.5 ± 0.033	7.0 ± 0.033	7.5 ± 0.1	6.0 ± 0.0	11.5 ± 0.0	11.5 ± 0.1
Streptococcus aureus 25923ATCC	7.0 ± 0.033	7.5 ± 0.067	7.8 ± 0.033	8.0 ± 0.033	6.0 ± 0.0	18 ± 0.167	19.3 ± 0.033
Enterococcus faecalis 29212ATCC	6.5 ± 0.058	6.5 ± 0.033	7.0 ± 0.033	7.5 ± 0.133	6.0 ± 0.0	13 ± 0.1	14.8 ± 0.033
Pseudomonas aeruginosa 27853ATCC	7.0 ± 0.033	7.5 ± 0.067	7.8 ± 0.067	8.3 ± 0.145	6.0 ± 0.0	20 ± 0.1	21.5 ± 0.1
Escheritia coli 25922ATCC	7.0 ± 0.0	7.5 ± 0.033	7.5 ± 0.033	8.0 ± 0.133	6.0 ± 0.0	19 ± 0.26	21.0 ± 0.115
Serratia marcscens (strain from local water)	7.5 ± 0.033	8. 5 ± 0.0	9.0 ± 0.067	9.0 ± 0.067	6.0 ± 0.0	21 ± 0.176	22.5 ± 0.088

Note. zone size includes 6mm well size and the result is mean of 3 values with ± SEM.

P.Ex. = plant extract, Strp. = Streptomycin, NC= negative control (DMSO)

Table 75. MIC and MBC investigated for *E. prostrata* in different bacterial species.

Bacterial Species	MIC (mg/ml)	MBC (mg/ml)
Klebsiella pneumonia 700603ATCC	10	50
Bacillus cerus QUI-ATCC	15	60
Citrobacter frundii QUF-ATCC	15	60
Streptococcus aureus 25923ATCC	10	40
Enterococcus faecalis 29212ATCC	10	50
Pseudomonas aeruginosa 27853ATCC	20	60
Escheritia coli 25922ATCC	10	50
Serratia marcscens (strain from local water)	5	30

Table 76. Inhibitory effect of *Euphorbia granulata* extract against ATCC fungal strains.

	Inhibition zones (mm)						
	Plant Extracts				Controls (+ ive and – ive)		
Bacterial Species	5mg	10mg	20mg	40mg	NC	10µg Fluc.	P.Ex.+ Fluc.
Aspergillus flavus QUF-ATCC	6.5 ± 0.1	7.0 ± 0.1	7.5 ± 0.115	7.8 ± 0.1	6.0 ± 0.0	20 ± 0.153	22.5 ± 0.115
Aspergillus niger QUI-ATCC	6.0 ± 0.115	6.5 ± 0.153	7.5 ± 0.2	8.6 ± 0.115	6.0 ± 0.0	13 ± 0.2	15.0 ± 0.115
Aspergillus parasiticus QUF-ATCC	7.0 ± 0.252	7.5 ± 0.153	7.8 ± 0.058	8.0 ± 0.2	6.0 ± 0.0	16 ± 0.115	18.5 ± 0.289
Fusarium oxysporum 1020ATCC	7.0 ± 0.0	7.5 ± 0.115	8.0 ± 0.2	8.3 ± 0.1	6.0 ± 0.0	13 ± 0.289	15.3 ± 0.115

Note. zone size includes 6mm well size and the result is mean of 3 values with ± SEM.

P.Ex. = plant extract, Fluc. = Fluconazole, NC= negative control (DMSO)

Table 77. Standard inhibition zone for antifungal activities (Seigismundo *et al*., 2008).

Zone size	Symbol	Results
≤ 6 mm	-	Negative
7-11 mm	+	Weak
12 – 16 mm	++	Moderate
≥ 17 mm	+++	Strong

Table 78. MIC and MFC investigated for *E. granulata* in different fungal species.

Bacterial Species	MIC (mg/ml)	MFC (mg/ml)
Aspergillus flavus QUF-ATCC	10	40
Aspergillus niger QUI-ATCC	10	30
Aspergillus parasiticus QUF-ATCC	10	40
Fusarium oxysporum 1020ATCC	10	40

Table 79: Inhibitory effect of *Euphorbia prostrata* extract against ATCC fungal strains.

	Inhibition zones (mm)						
	Plant Extracts				Controls (+ ive and – ive)		
Bacterial Species	5mg	10mg	20mg	40mg	NC	10µg Fluc.	P.Ex.+ Fluc.
Aspergillus flavus QUF-ATCC	7.0 ± 0.115	7.8 ± 0.115	8.5 ± 0.058	9.0 ± 0.115	6.0 ± 0.0	20 ± 0.289	22.5 ± 0.289
Aspergillus niger QUI-ATCC	7.0 ± 0.0	7.5 ± 0.058	8.0 ± 0.2	8.5 ± 0.115	6.0 ± 0.0	13 ± 0.289	16.0 ± 0.404
Aspergillus parasiticus QUF-ATCC	6.0 ± 0.0	6.5 ± 0.173	7.0 ± 0.115	7.5 ± 0.1	6.0 ± 0.0	16 ± 0.289	17.0 ± 0.404
Fusarium oxysporum 1020ATCC	7.0 ± 0.346	7.3 ± 0.115	7.6 ± 0.153	8.0 ± 0.404	6.0 ± 0.0	13 ± 0.231	17.5 ± .115

Note. zone size includes 6mm well size and the result is mean of 3 values with ± SEM.

P.Ex. = plant extract, Fluc. = Fluconazole, NC= negative control (DMSO)

Table 80. MIC and MFC investigated for *E. prostrata* in different fungal species.

Bacterial Species	MIC (mg/ml)	MFC (mg/ml)
Aspergillus flavus QUF-ATCC	5	30
Aspergillus niger QUI-ATCC	10	50
Aspergillus parasiticus QUF-ATCC	10	50
Fusarium oxysporum 1020ATCC	10	40

Table 81. Tumor suppressing effects of extracts from *E. granulata* using potato discs.

Ext. Conc. Per potato disc	Avg. no of tumors per disc	%age inhibition	LC_{50} (mg/disc)
1 mg	22 ± 2.236	(-10.00)	25
500 µg	22 ± 1.871	(-10.00)	25
250 µg	20 ± 2.0	0.00	25
125 µg	21 ± 1.581	(-5.00)	25
62.5 µg	19 ± 2.0	5.00	25
Vincristine 3.125 µg	0.0 ± 0.0	100	-
Negative control (DMSO)	20 ± 1.581	0	None

The results are the mean of five values ± SD

Table 82. Tumor suppressing effects of extracts from *E. granulata* using carrot discs.

Ext. Conc. Per potato disc	Avg no of tumors per disc	%age inhibition	LC_{50} (mg/disc)
1 mg	14 ± 2.0	6.67	25
500 µg	14 ± 1.871	6.67	25
250 µg	14 ± 1.581	6.67	25
125 µg	15 ± 2.0	0.00	25
62.5 µg	15 ± 1.581	0.00	25
Vincristine 3.125 µg	0.0 ± 0.0	100	-
Negative control (DMSO)	15 ± 1.0	0	None

The results are the mean of five values ± SD

Table 83. Tumor suppressing effects of extracts from *E. prostrata* using potato discs.

Ext. Conc. Per potato disc	Avg. no of tumors per disc	%age inhibition	LC_{50} (mg/disc)
1 mg	16 ± 1.225	15.79	20
500 µg	16 ± 1.871	15.79	20
250 µg	18 ± 2.236	5.26	20
125 µg	19 ± 1.871	0.0	20
62.5 µg	19 ± 2.449	0.0	20
Vincristine 3.125 µg	0.0 ± 0.0	100	-
Negative control (DMSO)	19 ± 2.739	0	None

The results are the mean of five values ± SD

Table 84. Tumor suppressing effects of extracts from *E. prostrata* using carrot discs.

Ext. Conc. Per potato disc	Avg no of tumors per disc	%age inhibition	LC_{50} (mg/disc)
1 mg	14 ± 2.0	(-27.27)	20
500 µg	12 ± 2.0	(-9.09)	20
250 µg	12 ± 2.55	(-9.09)	20
125 µg	11 ± 2.581	0.0	20
62.5 µg	11 ± 1.581	0.0	20
Vincristine 3.125 µg	0.0 ± 0.0	100	-
Negative control (DMSO)	11 ± 2.121	0	None

The results are the mean of five values ± SD

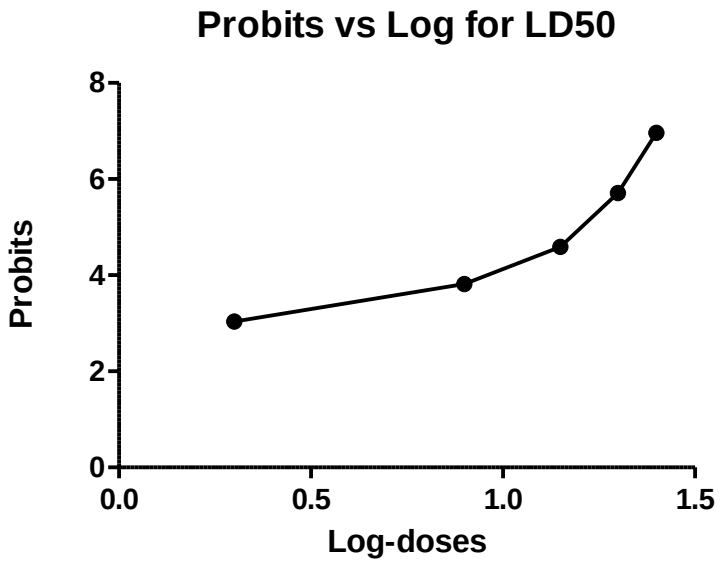

Fig. 22. LD_{50} determination for EG extract against brine shrimp larvae.

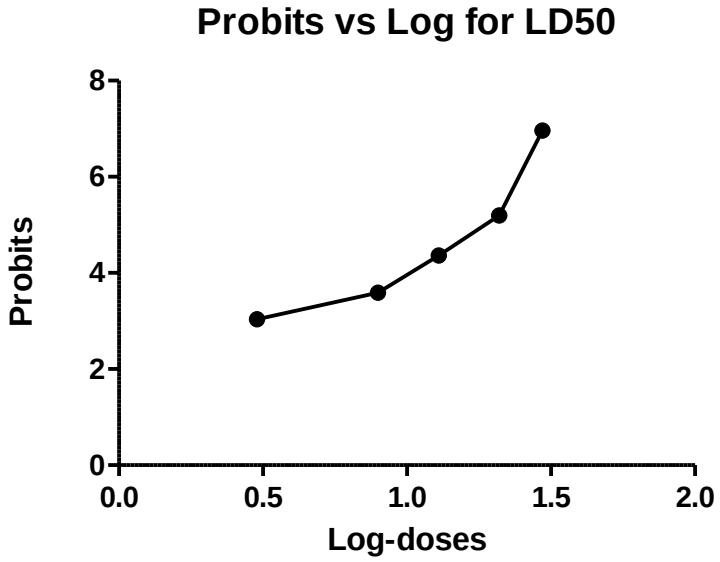

Fig. 23. LD_{50} determination for EP extract against brine shrimp larvae.

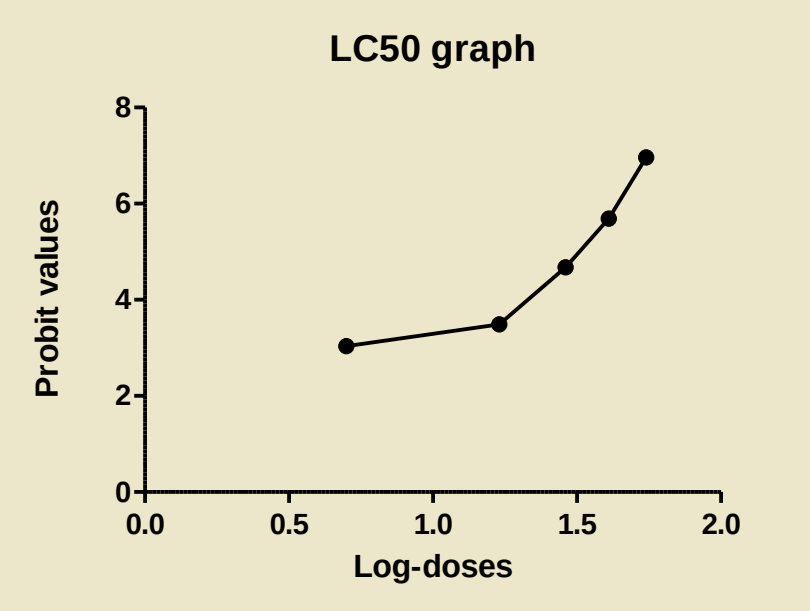

Fig. 24. LC_{50} determination for EG extract against *Lemna minor*.

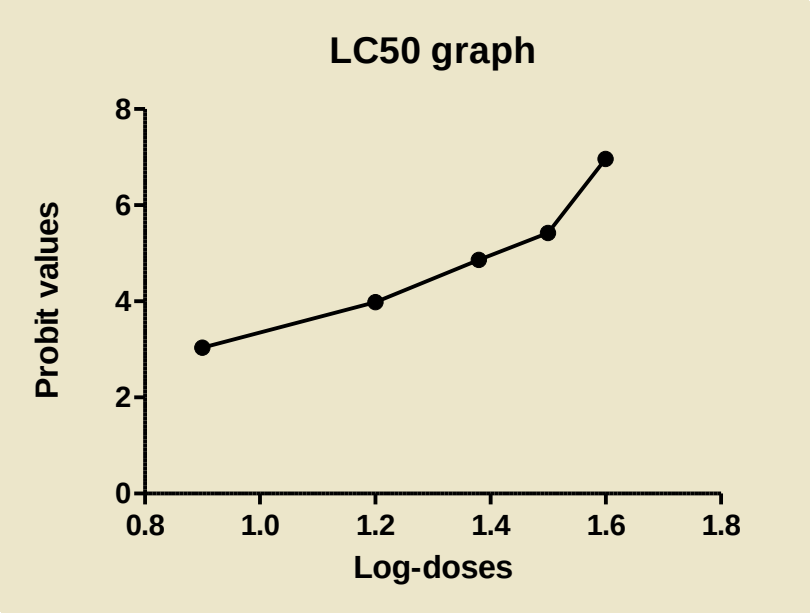

Fig. 25. LC_{50} determination for EP extract against *Lemna minor*.

CONCLUSION

- The two plants had excellent quantities of fibers (18.3-20%) proteins (17.5-18.3%), lipids (3.25-4.2%) and carbohydrates (32.3-40%). They had good amount of important phytochemicals including Flavonoids (0.5-0.7 mg/gm), Alkaloids (0.6-0.7 mg/gm), Tannin (0.2-0.3 mg/gm), Glycosides (0.6-0.9 mg/gm) and Saponins (0.2 mg/gm). Both micro and macro minerals were within the range of permissible limits.
- *Euphorbia granulata* was noted to extract Mo, Ni and As in amount larger than present in soil. On the other hand *Euphorbia prostrata* was noted for absorbing high quantities of Hg and Mo from soil. The overall concentration of heavy metals in the plant was not larger enough to produce toxicity.
- Both plants were noted for hypoglycemic properties but *Euphorbia granulata* was noted with fast action of reducing BGL. The hypoglycemic effects were more pronounced in normal than induced diabetic rabbits.
- *E. granulata* tend to control the level of TC, LDL and VLDL, however *Euphorbia prostrate* additionally had effect on FFA also.
- Both of them were also noted for improving the health of liver by repairing the damaged liver cell. It was further noted that the extracts of the two plants also protect the health.
- Treatment with extracts of both *Euphorbia granulata and Euphorbia prostrata* not only helped to activate osteoblasts activities but also enhanced calcium and phosphorus absorption. This was observed with increased serum levels of ALP, Ca and P.
- None of these plants had role in optimizing the serum electrolytes levels in rabbits.
- *Euphorbia granulata* was found not only to help in treating anemic conditions in rabbits but also help prevent the damage to RBCs. On the other hand *Euphorbia prostrata* was only effective in normalizing hematological markers anemic rabbits.

- There were no clear cut indications that plant either stimulate or suppress the immune system however some clues were found which shows that *E. prostrata* can increase the white blood cells count.
- *Euphorbia granulata* showed 59.93% (± 1.058) inhibition of DPPH was weak antioxidant compared to *Euphorbia prostrata* with 67.37% (±0.949) inhibition of DPPH.
- The two plant were not noted for cytotoxicity or phytotoxicity while *Euphorbia granulate* was noted for some stimulant effect on *Lemna minor*.
- No activity was noted against *Tribolium castaneum* either in *Euphorbia granulate* or *Euphorbia prostrata*.
- They had some role in inhibiting the growth of some selected bacterial and fungal species. While testing for antitumor activity the two plants were found ineffective.

RECOMMENDATIONS

- The two plants were noted with excellent nutritional profile. This profile can help supplementing important minerals nutrients and also be a source of essential amino acids (EAA), essential fatty acids (EFA) and vitamins in small amount. The plant grows easily and the growth can be enhanced with little efforts. Establishing the plant as a nutritional supplement may provide job opportunities and may boast the economy of the locals. The use of plant will also have positive impact on the health of its consumers.
- The presence of good quantity of phytochemicals and the antioxidant potential of plant makes it good choice for use in inflammatory conditions and prevention of hazard of free radicals. The plant can also be used as a source of quality antioxidants.
- The selective accumulation of heavy metals by the two plants recommend the plants to be used selectively were the soils are contaminated with Hg, Mo, Ni or As. Although the plant is small herb with small rooting system but the present advance in genetic engineering can make it possible to transfer the gene responsible for a particular metal to large size plants.
- The plant can be safely used for optimizing the levels of BGL and lipids and also for the liver disease. Once its use is established in these health related problems the plant can be produced on commercial scale for the economy of the locals. This will also help the locals in providing an economical, safe and effective remedy.
- The various therapeutic effects of the plant need further exploration of active principals which will pave the way for more research.
- The plant seems to have unique combination of phytochemicals, the identification and isolation of such molecules can be providing a base for developing many of isotypes with unique pharmacological impacts. The compounds so obtained can be used for other purposes as well.

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